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Investigation of Selected Dye Carriers Used in Commercial Dyeing of Hydrophobic Fibers

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Industrial Environmental Research Lab.
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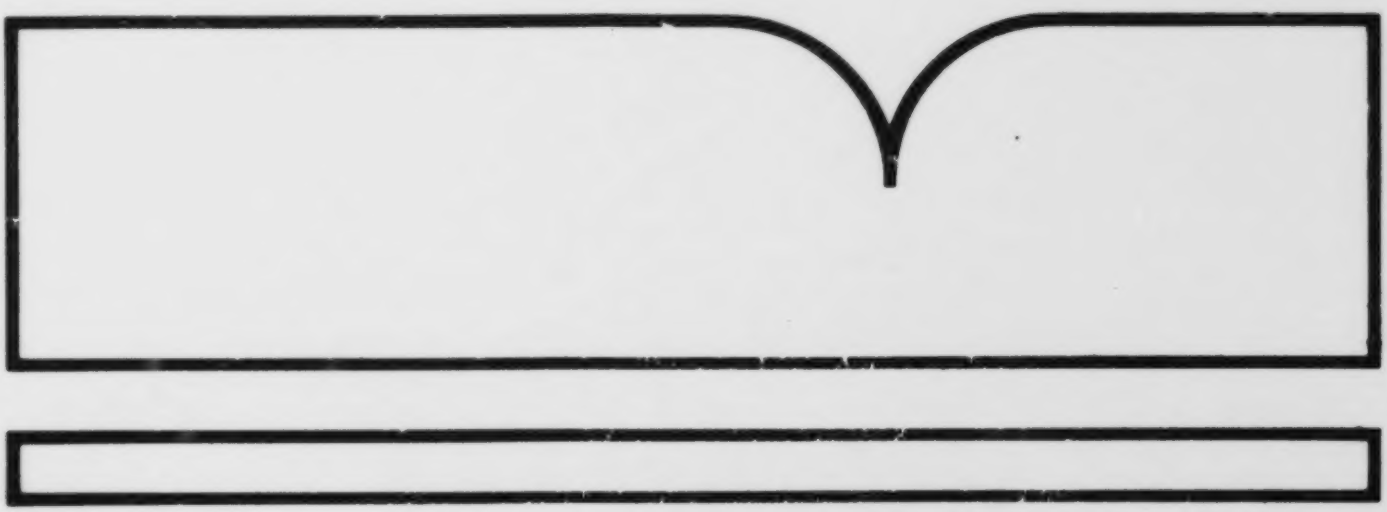

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AN INVESTIGATION OF SELECTED DYE CARRIERS USED IN COMMERCIAL DYEING OF HYDROPHOBIC FIBERS

by

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16. ABSTRACT The report gives results of an evaluation of toxicities of dyebath discharges from atmospheric dyeing of polyester fiber/fabric with three commercial dye carriers: trichlorobenzene (TCB), biphenyl (BP), and o-phenyl phenol (OPP). First, criteria were established to rank the priority of all commercial dye carriers, based on known toxicities and volumes of consumption. Commercial atmospheric dyeing of 100% polyester fiber/fabric was simulated in the laboratory, using a commercial disperse dye, other dyebath additives, and the selected dye carriers. Biological testing and chemical analyses were carried out on the discharges of these dyebath systems. Bioassays included: (1) Ames Salmonella/microsome mutagenesis assay, (2) acute in-vivo mouse quantal assay, and (3) aquatic ecological assay with Daphnia magna. Analytical procedures were established for both qualitative identification and quantitative determination of key dyebath components. In-depth studies were also carried out on two dyebath systems (TCB and BP) to identify species responsible for the observed toxicity and to delineate possible chemical and/or biological interactions between the dyebath components. The TCB system was also investigated to demonstrate that the toxicity of these dyebath discharges can be reduced significantly by increasing dyeing efficiency.

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a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Pollution	Pollution Control	13B
Bioassay	Stationary Sources	13H
Dyeing	Dye Carriers	11C
Dyes	Hydrophobic Fibers	06T
Toxicity	Trichlorobenzene	11E
Polyester Fibers	O-phenyl Phenol	07C
Biphenyl		06A

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ABSTRACT

Toxicities of dyebath discharges from atmospheric dyeing of polyester fiber/fabric with three different commercial dye carriers were evaluated. Criteria were first established to rank the priority of all commercial dye carriers, based on known toxicities and volume of consumption. Three commercial dye carriers, trichlorobenzene (TCB), biphenyl (BP) and o-phenyl phenol (OPP), were selected for study. Commercial atmospheric dyeing of 100% polyester fiber/fabric was simulated in the laboratory, using a commercial disperse dye, other dyebath additives and each of the selected dye carriers.

Biological testings and chemical analyses were carried out on the discharges of these dyebath systems. Bioassays included 1) Ames Salmonella/-Microsome Mutagenesis assay, 2) acute IN VIVO Mouse Quantal assay and, 3) Aquatic Ecological assay with Daphnia magna. Analytical procedures were established for both qualitative identification and quantitative determination of key dyebath components.

In-depth studies were also carried out on two dyebath systems, trichlorobenzene and biphenyl, to identify species responsible for the observed toxicity and to delineate possible chemical and/or biological interactions among the dyebath components.

An investigation was also made on the trichlorobenzene system to demonstrate that the toxicity of these dyebath discharges can be reduced significantly by increasing dyeing efficiency. The dyeing process was carefully evaluated to determine the dye and carrier contents necessary to achieve maximum dyebath efficiency.

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SECTION 1

INTRODUCTION

The Clean Water Act of 1977 identified 65 specific toxic compounds and classes of toxic compounds which are subject to effluent limitations and "expanded the spectrum of pollutant parameters to receive attention in point source discharges to include potentially toxic pollutants".[1] Within these 65 classes, specific elements or compounds have been identified as priority pollutants. Included in this priority list of 129 compounds are 13 metals, 114 organic compounds, cyanide and asbestos.[2]

Wastewater guidelines for the textile industry were based on studies sponsored jointly by EPA and the industry. The water use and wastewater discharge statistics for the textile industry were reported in detail by Sverdrup and Parcel and Associates, Inc. in a study conducted for EPA.[1] This study indicates that the dyeing step in the finishing of woven and knitted fabrics and in carpet finishing is responsible for much of the waste volume and contributes substantially to the waste load in the finishing process. Color is an obvious adverse characteristic of the wastewater along with high levels of dissolved solids. Carriers used in dyeing of polyester (and other hydrophobic fibers) contribute significantly to the biochemical oxygen demand (BOD). Carriers which are used to accelerate the dyeing of hydrophobic fibers with disperse dyes (and sometimes other classes of dyes) are of particular concern in the dyeing waste stream. Since some of them appear as specific chemicals on the EPA Priority Pollutant List.[1]

The present study has focused on the laboratory investigation of the toxicity of major dye carriers currently used in the commercial dyeing of polyester fiber/fabric. The study includes development of analytical techniques and sytematic biological evaluations necessary to identify and quantify toxic species in these systems. The study was also intended to provide information which can be used to eliminate or to reduce toxic substances in textile industry waste discharges.

The systematic approach taken to carry out the above objectives was as follows:

i) Established priority ranking of current commerical dye carriers for polyester fiber/fabric, based on available data on current consumption and toxicity of these chemicals,

ii) carried out biological and chemical screening on the three top ranked carrier systems, according to the order of the priority established,

iii) carried out in-depth biological and chemical studies on the first two toxic systems identified in the above screening studies, the objective of these in-depth studies was to identify the species responsible for the observed toxicity and to delineate possible interaction (synergistic or antagonistic) among species in the system and,

iv) investigated possible means of eliminating or reducing toxicity of these systems by varying dyebath contents, dyebath conditions and/or dyeing procedures.

SECTION 2

CONCLUSIONS

Toxicity screening of spentbath samples from laboratory-simulated atmospheric dyeing of 100% polyester fabric with a commercial disperse dye (C.I. Yellow 23) and commerical dye carriers, trichlorobenzene (TCB), biphenyl (BP) and o-phenyl phenol (OPP), indicated that all three carrier systems are extremely toxic to Daphnia magna assay. Median effective concentrations, EC_{50}, for the 48 hrs. test were 25.5%, 9.28% and 0.91%, respectively for the TCB, BP and OPP carrier systems. However, results of Ames Salmonella/-Microsome Mutagenisis assay and acute IN VIVO Mouse Quantal assay were negative for all three carrier systems.

In-depth studies carried out on the TCB and BP carrier systems, showed that toxicity in these two systems was due mainly to the dye (Yellow 23) and the carrier (TCB or BP), no other dyebath additives were found to have any significant toxicity. Furthermore, the toxicity observed in these sytems was found to be the additive effect of the dye and the carrier. In-depth study of the OPP System (the most toxic to Daphnia magna) was not conducted due to budget constraints in the project.

A material balance made around the dyeing process showed that, in all three systems (TCB, BP and OPP), significant amount (more than 50%) of the original carrier content in the dyebath was lost through volatilization during dyeing. This may have an adverse impact on the air environment.

Studies varying dyeing time and original carrier content indicate that dyeing conditions can be optimized to minimize the amount of residual dye and

carrier in the wastewater. This is one potential technique for reducing the toxicity of these wastewaters that should be considered.

The present study has developed and established a basis for analytical techniques which can be applied to relatively simple textile waste systems, such as dyebath discharges. It has also demonstrated that the systematic approach of biological and chemical screening and in-depth investigation is an effective means of identifying toxic species and evaluating overall toxicity of these systems. However, successful applications of the techniques and the approach to a more complex textile waste discharge remains to be demonstrated.

SECTION 3

MATERIALS USED IN STUDY

SELECTION OF SYSTEMS

Selection of candidate systems, including fiber/fabric and dyeing process, dye, dye carrier and other dyebath additives are discussed below. A review of known biological activity of these systems is also presented in this section.

Fiber/Fabric and Dyeing Process

Hydrophobic fibers such as polyester, nylon and acrylics require carriers in the dyeing process. Due to the extremely high volume of polyester fiber produced (45% of the total U. S. man-made fiber production in 1978) and even higher proportion of polyester fiber in the apparel market, relative to other hydrophobic fibers, this study dealt mainly with the dyeing of polyester fiber/fabric. Polyester can be dyed as fiber, yarn or fabric (both 100% polyester and polyester/cellulosic blends), and can be dyed under atmospheric conditions, pressured conditions or by the thermosol technique. Atmospheric dyeing requires a higher carrier concentration in the dyebath, about 8% as compared to 3% for pressured and 2% for thermosol dyeings. Thus, in this study we selected to investigate the conditions of atmospheric dyeing of 100% polyester woven fabric due to the higher carrier concentration required. The majority of polyester/cellulosic blend fabrics (about 85%) are dyed with the thermosol technique which requires a much lower concentration of carriers. With the large volume of polyester/cellulosic blends produced, the overall environmental impact of the thermosol process may be significant and should

not be ignored. However, the dyeing of polyester fabric with the atmospheric process should be investigated first, due to the following reasons;

1) high concentration of carriers required in this process, the results should reflect the maximum toxic level in the textile waste water,
2) carriers used in the dyeing of polyester/cotton blends (thermosol) are the same as those used in atmospheric process for polyester,
3) the atmospheric process, with single dyestuff, has relatively simple dyebath conditions. The results of this study, particularly those of the chemical study, should provide a basis for future investigation of other processes involving complex dyebath conditions such as the thermosol process for blends.

The fabric selected was a light weight 100% polyester woven fabric obtained from a manufacturer.

Commercial Carriers

Criteria for ranking the priority of commercial dye carriers to be studied were established as follows:

a) EPA Priority Pollutant List - toxic pollutant found most frequently in textile wastewater,
b) known toxicity (oral-rats LD_{50}) of carriers and,
c) consumption volume of carriers in the U.S.

The priority rankings of all commercial dye carriers were made based on the following procedure:

i) all commercial dye carriers used in the U.S. for polyester fiber/fabric were compiled and identified by major chemical composition,

ii) data on U.S. industrial consumption of these chemicals as dye carriers were obtained,

iii) known toxicities (LD_{50}) of these chemicals were identified from the NIOSH data;[4] information regarding the bio-degradability of these chemicals was also obtained,

iv) a formula taking into consideration the consumption volume and toxicity data (i.e., consumption/LD_{50}) was established to rank all commercial dye carriers,

v) carriers obtaining similar ranking according to the above formula were then ranked by their resistance to bio-degradation (chemical with low bio-degradability will be ranked as a higher priority).

A total of 18 chemicals were identified as a major component of commercial dye carriers used in the U.S. The consumption volume in 1975 and LD_{50} for these chemicals have also been obtained (Table I). Information from the industry and other sources indicated that there was no significant change in the relative volume of usage of most of these chemicals as dye carriers in 1980. Information, however, indicated that there were significant reductions in the usage of perchloroethylene, naphthalene and methyl naphthalene as dye carriers. A factor considering both the volume and the toxicity (Column 3, Table I) was used for initial ranking of these chemicals. Among the 18 chemicals, five (5) are on the EPA Priority Pollutant List, whereas three (3) of these five are also among the list of the toxic pollutants "found most frequently in the textile wastewater." Four (4) candidate dye carriers were selected and their priority rankings for investigation were obtained as follows:

TABLE I. COMMERCIAL DYE CARRIERS (U.S.)

	Volume of Consumption,[a] 10^6 lb.	LD_{50}[b]	$(Vol/LD_{50}) \times 10^3$
biphenyl	53	3280	16.16
methyl naphthalene	13[c]	5000	2.60
o-phenyl phenol	15	4100	3.66
butyl benzoate	11	5140	2.14
trichlorobenzene[d,e]	5	756	6.61
perchloroethylene[d,e]	5	8850	.56
napthalene[d,e]	1.6	1780	.90
methyl benzoate	1.7	1350	.96
methyl p-toluate benzoate	1.7	-	
methyl biphenyl	3.5	-	
methyl cresotinate	.9	-	
o-phenyl oxide	.9	3990	.23
benzyl benzoate	.9	1770	.52
dimethyl phthalate[d]	.9	6900	.13
methyl salicylate	.9	887	1.01
chloro-o-phenyl pehnol	.7	4220	.17
dichlorobenzene[d]	.7	500	1.40
benzaldehyde	.7	1300	.54

a. U.S. consumption (as dye carrier), 1975

b. Taken from "Registry of Toxic Effects of Chemical Substance, 1978," NIOSH, Oral-rats.

c. Possible significant change (reduction in current usage.

d. On EPA Priority Pollutant List.

e. On EPA Pollutant List of "Found Most Frequently in Textile Wastewaters."

1) trichlorobenzene

2) biphenyl

3) o-phenyl phenol

4) butyl benzoate

Screening studies were carried out on the trichlorobenzene, biphenyl and o-phenyl phenol systems. In-depth studies were done on the trichlorobenzene and biphenyl systems.

Commercial Dyes

Three disperse dyes were first selected as candidate dyestuffs for this study, based on their volume of sale in the U.S. (Table II). Disperse yellow 23 was finally selected as the dye to be used throughout this study. The selection was based on the relatively simple chemical structure of this dye and its availability in pure form (through synthesis and/or purification). Two commercial samples of this dye were obtained from two different sources.

MATERIALS

Commercial Dye and Carriers

All dye, carriers and other dyebath additives were commercial formulations obtained directly from the suppliers. Compositions of these commercial samples are shown in Table III.

Reagents and Solvents

Spectro-grade solvents were used for all Ultraviolet-Visible (UV-VIS) and Infrared (IR) spectrophotometric analyses. High Performance Liquid Chromatograph (HPLC) grade solvents were used for all liquid chromatographic analyses. All other chemicals and solvents used were reagent grade.

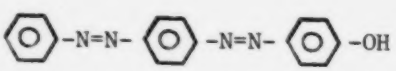
Table II. U.S. Sales and Characteristics of Selected Disperse Dyes

U. S. SALES:

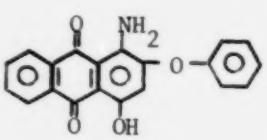
Dye	U.S. Sales(1978), 1,000 lb
Disperse Yellow 23	571
Disperse Red 60	1,789
Disperse Blue 79	3,310
Total Disperse Dyes	39,721

CHARACTERISTICS:

Disperse Yellow 23 (Chemical Class - Disazophenol)



Disperse Red 60 (Chemical Class - Anthraquinone)



Disperse Blue 79 (Chemical Class - Monoazo)

(Chemical Structure Unknown)

TABLE III. COMMERCIAL FORMULATIONS OF DYEBATH ADDITIVES

Chemical	Trade-Name	Composition	Source
Carrier:			
Trichlorobenzene		93% TCB (isomers) 7% emulsifier[a]	American Color & Chemicals
Biphenyl	Carolid RNO	30% biphenyl 50% chlorotulene 15% petroleum naphtha	Tanatex
O-Phenyl Phenol	Carolid LP	40% o-phenyl phenol 3% butyl cellosolve 7% petroleum fractions	Tanatex
Dye:			
Disperse Yellow 23		48 parts yellow 23 80 parts dispersing agent[b] 2 parts oil	
Wetting Agent:			
Anionic	Amaterg T.	Sodium n-methyl-n-oleoyltaurate	American Color & Chemicals

[a] [b] Contains methylalcohol, aromatic petroleum solvent, alkyl aryl sulfonate and nonionic surfactant

[c] Reax 83A contains ligninosulfonate

SECTION 4

EXPERIMENTAL PROCEDURES

The general approach of this study was 1) to carry out screening studies on the selected commercial systems to determine the toxicity of these systems and then, 2) to follow up with in-depth analyses on the toxic systems, to identify the species responsible for the observed toxicity and to delineate possible mechanisms of formation of the toxic species.

DYEING AND SAMPLING PROCEDURES - SCREENING

A dyebath composition and dyeing procedure, simulating that of industrial atmospheric beck dyeing of 100% polyester, is outlined in Table IV. This dyeing procedure was used throughout this study in preparing dyebath samples for screening studies.

Three consecutive dyebaths were prepared. Four (4) fabric samples of equal weight were dyed in each bath, as shown in Fig. 1. At the completion of each dyeing process, two (2) fabric samples were pulled out of the bath and each placed in a Soxhlet for extraction with $MeCl_2$ to determine the wet pick-up. The other two (2) fabric samples were then pulled out of the bath and subjected to scouring and heat curing. One of these two finished samples was again extracted with $MeCl_2$ in a Soxhlet to determine the amount of dye and carrier on the fabric (dry add-on).

After adjusting pH (with acetic acid) and volume of each spentbath liquor, 350 ml. of liquor was taken from each bath and stored individually for

TABLE IV. DYEING PROCEDURE

Dye Bath:

Bath Ratio (liquid:fabric)	30:1 - 40:1
Carrier	3.5 g/l
Wetting Agent (anionic)	0.4% OWF
Acetic Acid	pH. 4.5 - 5.0
Dyestuff (Disperse yellow 23)	0.7% OWF
Water (distilled)	balance

Procedure:

1. bath make-up -- water, wetting agent, carrier and adjust pH. 4.5-5.0 with acetic acid
2. heat bath to 120° F.
3. add fabric with stirring and heat to 150° F.
4. add dyestuff and cover the bath
5. raise temperature to 180° F. with stirring at the rate of 3-5° F/min.
6. slow temperature increase to 1° F/min at 185°-195° F. range
7. raise temperature to boiling and boil for 45-60 minutes. (Maintain volume through process by adding boiling water)
8. cool at 160° F.
9. dump
10. scour fabric at 160° F.
11. rinse with hot wat er (distilled) until clear

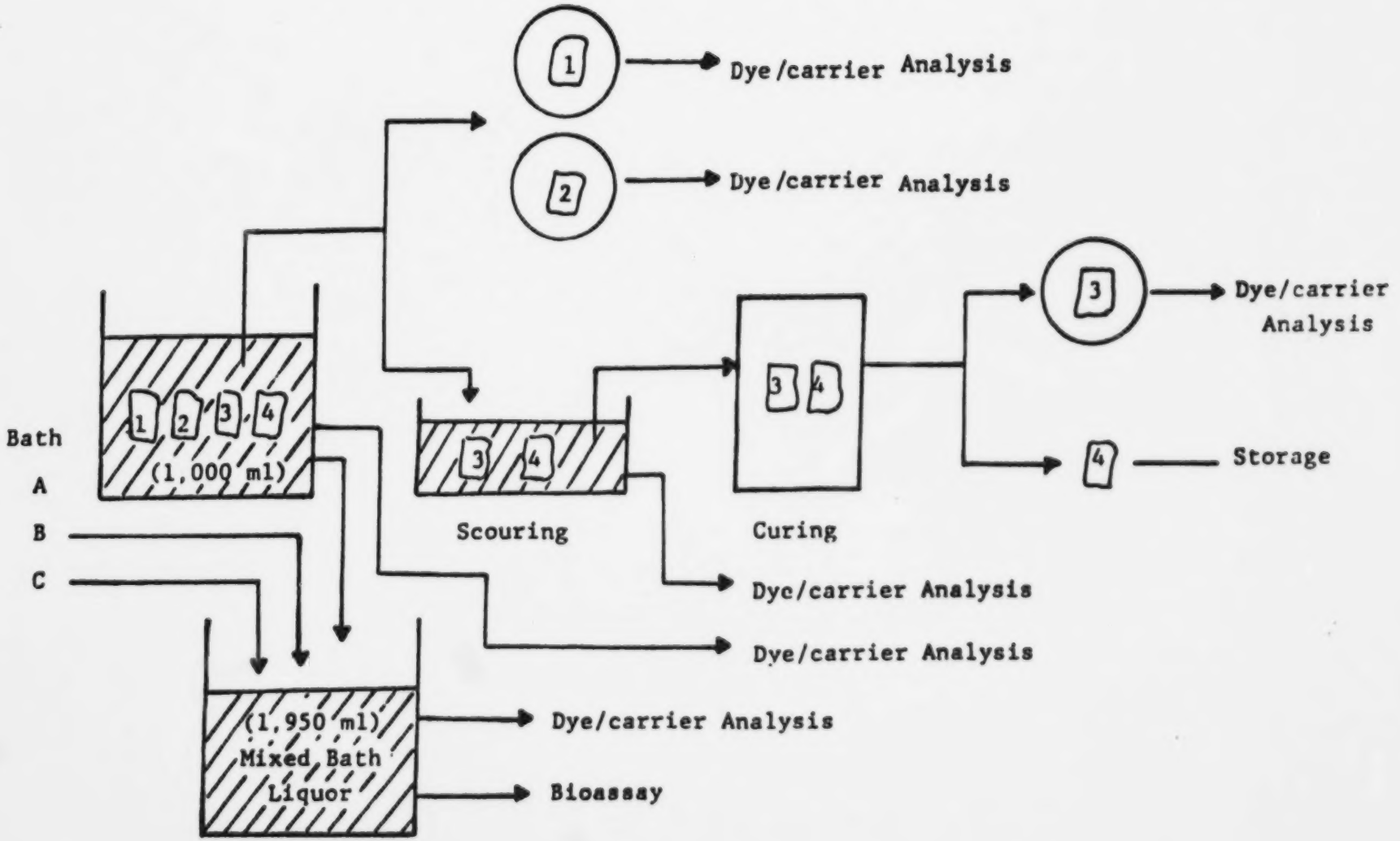


Figure 1. Schematic Procedure of Dyeing and Analyses.

chemical analyses. The balance of each volume (650 ml) was mixed. An aliquot of 350 ml. of liquor was taken from this mixed bath for chemical analyses and the rest (1,600 ml.) was sent for bioassay.

IN-DEPTH STUDIES

The general procedure used in the in-depth studies was as follows:

i) Toxicities of major components (dye and carrier) in the screening dyebath sample were evaluated individually, samples containing individual components at the concentration identical to that in the dyebath sample were prepared, and bioassay results were obtained on these samples.

ii) Where it was difficult to prepare samples containing individual components due to low solubility, etc., toxicity of the sample containing commercial formulation was first evaluated. For example, due to poor solubility in water, it was impossible to prepare carrier-containing samples at the concentration comparable to those of the dyebath samples. A sample containing commercial carrier formulation (carrier + emulsifier) was then evaluated and then a sample containing emulsifier alone was also evaluated in order to elucidate the toxicity of the carrier.

iii) After the toxicity of the major component was evaluated, other components in the dyebath were then added one at a time, and the toxicity of the sample was re-evaluated after each addition of a component, if necessary.

This procedure allowed identification of the species responsible for the observed toxicity of dyebath sample with the minimum number of bioassay testings. It also allowed delineation of possible synergistic or antagonistic effects in the dyebath samples.

BIOASSAY PROCEDURES

Biological assessment of toxicity was done on liquid discharges from all screening and in-depth studies. Bioassay testings included two (2) tests in health effects and one (1) in aquatic ecological effects, as follows:

Health Effects Tests:

a) Salmonella/Microsome Mutagenesis Assay (Ames)

b) Acute IN-VIVO Test in Mouse (LD_{50})

Aquatic Ecological Effects Test:

a) Acute Static Bioassay with Freshwater Invertebrate-Daphnia Magna Assay.

For screening studies, all these bioassay tests were carried out. However, the test which showed most sensitivity (highest toxicity) was followed in the subsequent in-depth study.

All bioassay testings were carried out according to the protocols used by EPA for environmental assessment. Litton Bionetics, Inc. (Kensington, Maryland) was awarded the contract by the University of Maryland to carry out the Ames Salmonella/Microsome Mutagenesis Assay and Acute IN VIVO Assay on mice. The Aquatic Ecological Assays in Freshwater Invertebrate-Daphnia magna were carried out by the Battelle Lab. (Columbus, Ohio), under subcontract from Litton Bionetics. Protocols and procedures for each of the above bioassays are described below.

Ames Salmonella/Microsome Mutagenesis Assay

The Ames assay is based on the property of selected Salmonella typhimurium mutants to revert from a histidine requiring state (auxotrophy) to an histidine synthesizing state (prototrophy) as a result of exposure to mutagens (5). The test is designed to mimic mammalian metabolic processes by the incorporation of a mammalian liver 9,000 X g microsomal fraction (S-9) and the cofactors necessary to generate enzymatic activity. The test is used as a screen for mutagenic activity of pure compounds, complex mixtures or component fractions.

The indicator organisms to be used are the Salmonella typhimurium tester strains developed by B. Ames.[5] They are histidine-deficient variants and are used to detect reverse mutations. The activation system for mutagenesis screening consists of Aroclor 1254 induced S-9 fraction derived from rat livers. Male, Sprague-Dawley rats weighing about 200 g each are used. Induction is accomplished by a single intraperitoneal injection of Aroclor 1254 (diluted in corn oil to 200 mg/ml) into each rat 5 days before sacrifice at a dosage of 0.5 mg/g of body weight. All rats are deprived of food (not water) 12 hours before sacrifice. The following steps are carried out at 4°C using cold sterile solutions and glassware.

The livers (10 to 15 g) are aseptically removed from the rats and placed into a cold preweighed beaker containing 10 to 15 ml of 0.15M KCl. After the livers are washed and weighed in this beaker, they are removed with forceps to a second beaker containing 3 ml of the KCl solution per gram of wet liver weight. The livers are then minced with sterile scissors, transferred to a chilled glass homogenizing tube, and homogenized in an ice bath by passing a

low-speed motor-driven pestle through the livers a maximum of three times. The chilled homogenates are then placed into centrifuge tubes and centrifuged for 10 minutes at 9,000 X g at 4°C. The resulting supernatant is decanted, transferred in 2-ml amounts to small storage tubes, quickly frozen in dry ice, and stored in -75° to -80°C in a low temperature freezer. This supernatant is known as the S-9 fraction. Sufficient S-9 for use each day is thawed at room temperature and kept on ice before and during use. The extent of bacterial contamination of the S-9 fraction should be determined. The S-9 mix may be filtered sterilized (0.45 m porosity filter) if required.

The quality of each S-9 lot is determined before the lot is released for general use. The enzymatic activity of S-9 is measured by testing reference mutagens, such as benzo-a-pyrene or 2-anthramine, in the Ames assay. These chemicals require metabolic activation to be detected as mutagens. The protein content of the S-9 is also determined. The normal range of values is 28 to 45 mg of protein per milliliter of S-9.

In the plate incorporation assay, the sample is added directly to molten top agar (45°C) which is then poured onto the plates along with the test organism and the liver S-9 activation system. Test samples are diluted in water, dimethylsulfoxide, ethanol, or acetone so that constant volume aliquots are added to each plate. Maximum liquid volume of test sample and vehicle should be about 0.5 ml per plate. Once the overlay has solidified, the plates are incubated at 37°C for 36 to 72 hours. Plates are then scored for the number of revertants per plate.

It is recommended that the standard plate incorporation assay be performed in duplicate for each test concentration and control. Controls for each

test include solvent or vehicle controls to measure the spontaneous reversion frequency for each tester strain. A sterility check of the test material solution or suspension is made. Positive controls consisting of compounds which both do and do not require metabolic activation are also run concurrently with each assay.

Testing strategy and dose selection are dependent upon sample type. Liquid samples are tested on a weight per volume basis. If the test is negative, it should be repeated after 1 week. In special cases, organic extracts may be made of particular samples for testing.[6] If the results are positive, repeat studies shall be performed over a narrower concentration range with strains showing positive results in the initial test to obtain does response curves. Solids may be tested up to 10 mg per plate if needed.

All liquids are initially tested in a minimum assay starting at the MAD concentration of 200 1/ml per plate, and at lower concentrations of 100, 50 and 1 1/ml. Nonaqueous sample are retested after 1 week if the test is negative. The samples are retested over a narrower range of concentration with strains showing positive results initially.

Acute IN VIVO Rodent Assay

Random bred weanling mice (21 to 28 days old) are used for Level 1 acute in vivo roden tests. Weanlings are used because they may be more sensitive to toxic effects of test materials than adult mice. In addition, significantly less test material is required for dosing.

Since the major objective of the Level 1 biological testing procedure is to identify toxicological problems at minimal cost, it is recomended that a two-step approach be taken to the initial acute in vivo toxicological evalua-

tion of unknown compounds. The first is based on the quantal (all-or-none) response and the second on the quantitative (graded) response. Normally, the quantal test is used to determine the necessity for carrying out the quantitative assay. If no animals die in the quantal test, further in vivo testing is not initiated and the sample toxicity is categorized as not detectable (ND).

Quantal --

Five male and five female weanling random bred mice are used for each dose level or control. The weight of each animal is determined before dosing. The test material is administered by gavage to this population of animals in three fractionated doses within 8 hours, to give a total dose of 10 ml/kg for liquids and 10 gm/kg for solids (solubilized or suspended in a suitable vehicle).

Immediately following administration of the test substance and at frequent intervals during the first day, observations of the frequency and severity of all toxic signs or pharmacological effects (Table 1) are recorded on an observation checklist (3). Particular attention is paid to time of onset and disappearance of signs. Daily observations are made and recorded on all animals through a 14-day period. At termination of the observation period, all surviving animals are weighed, killed, and then gross necropsies performed. Necropsies are also performed on all animals that die during the course of this study.

Quantitative --

If a single animal in the quantal study dies in the 14-day observation period, a quantitative study is performed. Forty weanling mice, equally divided by sex, are used for this study. After determining good health in the

study population following 7 days of quarantine, the animals are randomly divided into four groups of ten animals (five of each sex). The test substance is administered in graded dosages according to the following schedule: 3.0, 1.0, 0.3, and 0.1 g/kg or ml/kg. A different dosage schedule may be selected depending on the results of the quantal study. The observations, study duration, and necropsy procedures are carried out as described for the quantal test.

Level 1 Aquatic Ecological Assay (Daphnia Magna)

Biological responses and bioaccumulation as well as chemical and physical parameters must be considered when assessing the potential impact of complex wastes on the aquatic environment. Biological testing for aquatic ecological effects usually consists of static acute toxicity tests on selected organisms representative of the various trophic levels. Evaluation of the bioaccumulation of components in complex mixtures is accomplished using a laboratory technique for simulating bioaccumulation phenomena.

Acute toxicity tests are used to determine the concentration of test material that produces an adverse effect on a specified percentage of the test organisms in a short period of time. Because mortality is normally an easily detected and an obviously important adverse effect, the most common acute toxicity test is the acute lethality test. The index most often used with fish is the 96-hour median lethal concentration (96-hour LC_{50}) and for macroinvertebrates the 48-hour effective concentration (48-hour EC_{50}). The LC_{50} is a statistically derived estimate of the concentration of toxicant in dilution water that is lethal to 50 percent of the test organisms during continuous exposure for a specified period of time, based on data from

one experiment. This may be supplemented, in fish tests with effects on behavior. The EC_{50} for macroinvertebrates is an estimate of the concentration of test material that results in the immobilization of 50 percent of the test organisms during continuous exposure for a specified period of time in one experiment. Immobilization is defined as lack of movement except for minor activity of appendages. This measured effect is used because death is not always easily determined with some invertebrates.

The static toxicity tests with Daphnia provide a large amount of data in a short period of time. The recommended test organisms are the early instars of Daphnia magna. The static acute exposure period for the Daphnia is 48 hours. Because death is not always easily determined in Daphnia, the 48-hour effective concentration (48-hour EC_{50}) is calculated for Daphnia.

Daphnia magna are maintained in a static system at 19-22°C. Tanks must be siphoned periodically to remove debris and water added as necessary to maintain volume. Cultures must be maintained under optimum conditions at all times to prevent formation of ephippial eggs; daphnids from cultures in which ephippia are being produced must not be used in testing.

The organisms should be fed at least once a day, at which time careful observations should also be made for mortality and for signs of disease, stress, and injury. Dead and abnormal individuals should be removed as soon as they are observed.

Water quality should be held constant as described above and temperature changes should not exceed 3°C in any 12-hour period.

The organisms should be handled as little as possible. When handling is necessary, it should be done as gently, carefully, and quickly as possible so

that the organisms are not needlessly stressed. After collection or transportation, the Daphnia should be held in and acclimated to the dilution water for at least 2 days before beginning a test. A minimal criterion for an acceptable dilution water is that healthy organisms will survive in it for the duration of acclimation and testing without showing signs of stress such as discoloration or unusual behavior. Water in which daphnids will survive and reproduce satisfactorily should be an acceptable dilution water for tests with freshwater organisms.

Unless the approximate toxicity of the sample is already known, at least six concentrations of test material should be prepared. The highest dose should be at the maximum applicable dose (MAD) for that sample type unless physical characteristics of the sample or other previously gathered toxicity data contravenes this. The concentrations should be in a geometric series, and each one should be at least 50 percent of the next higher one.

For Daphnia magna tests, five organisms per replicate with three replicates per concentration should be used. The use of more organisms and replicate test containers and random assignments of test organisms to containers is desirable.

The Daphnia tests should be conducted at 19 $\pm$ 2°C. A photo period of 16 hours light and 8 hours dark is used. Organisms should not be fed during exposure. The test conditions are summarized below.

Temperature, °C	19 ± 2
Photoperiod, hours light:dark	16:8
Water quality, hardness mg/l as $CaCO_3$	100
Container size	250 ml
Test volume	200 ml
Organisms per container	5
Replicates	3
Feed	No
Duration, hours	48
Measurements of D.O. and pH, hours	0, 48

Each test requires a control which consists of the same dilution water, conditions, procedures, and organisms as used in the test concentrations. If any solvent other than water is present in the test concentration, a solvent control is also required. This solvent control is treated the same as the control except that the highest amount of solvent present in any other test containers is added to this test container.

In the daphnid test there should be 2 to 3 liters of solution or control water in each 3.9 liter wide-mouth bottle or 3.3-liter battery jar, or 200 milliliters in each 250-milliliter beaker.

Test organisms should be placed in the test and control vessels not more than 30 minutes after the test solutions are prepared. Five daphnids in each replicate are recomended. Dissolved oxygen, pH, and conductivity (when

required) on the high, medium and low concentrations, and temperature should be recorded initially and at 48 hours.

Mortality is the effect most often used to define acute toxicity to aquatic organisms. Because death is not always easily determined with some invertebrates, an EC_{50} is calculated. The principal criterion for effect on Daphnia is immobilization, defined as lack of movement except for minor activity of appendages.

Mortality or immobilization and abnormal behavior should be recorded every 24 hours for the duration of the test. Dead or immobilized organisms should be removed as soon as they are observed.

The concentration of test material effective (causing immobilization) to 50 percent of the population (EC_{50}) and 95 percent confidence limits should be determined at 24- and 48-hour exposures. Any of several methods including moving average, Spearman Karber, Litchfield-Wilcoxin, probit, or binomial may be used.

GENERAL ANALYTICAL PROCEDURES

Various analytical techniques and instrumentations were employed in the analysis, both qualitative and quantitative, of dyebath samples, dyed fabrics and other liquid samples prepared in the screening and in-depth studies. Figure 2 shows a flowchart of the procedure used for preparing screening samples (dyebaths and fabrics) for various analyses.

Dyebath Solution Extraction

A 100 ml aliquot of the dyebath solution was extracted in a separatory funnel with 25 ml of methylene chloride. The solution was shaken for 3 minutes, anhydrous magnesium sulfate was added and the solution was allowed to

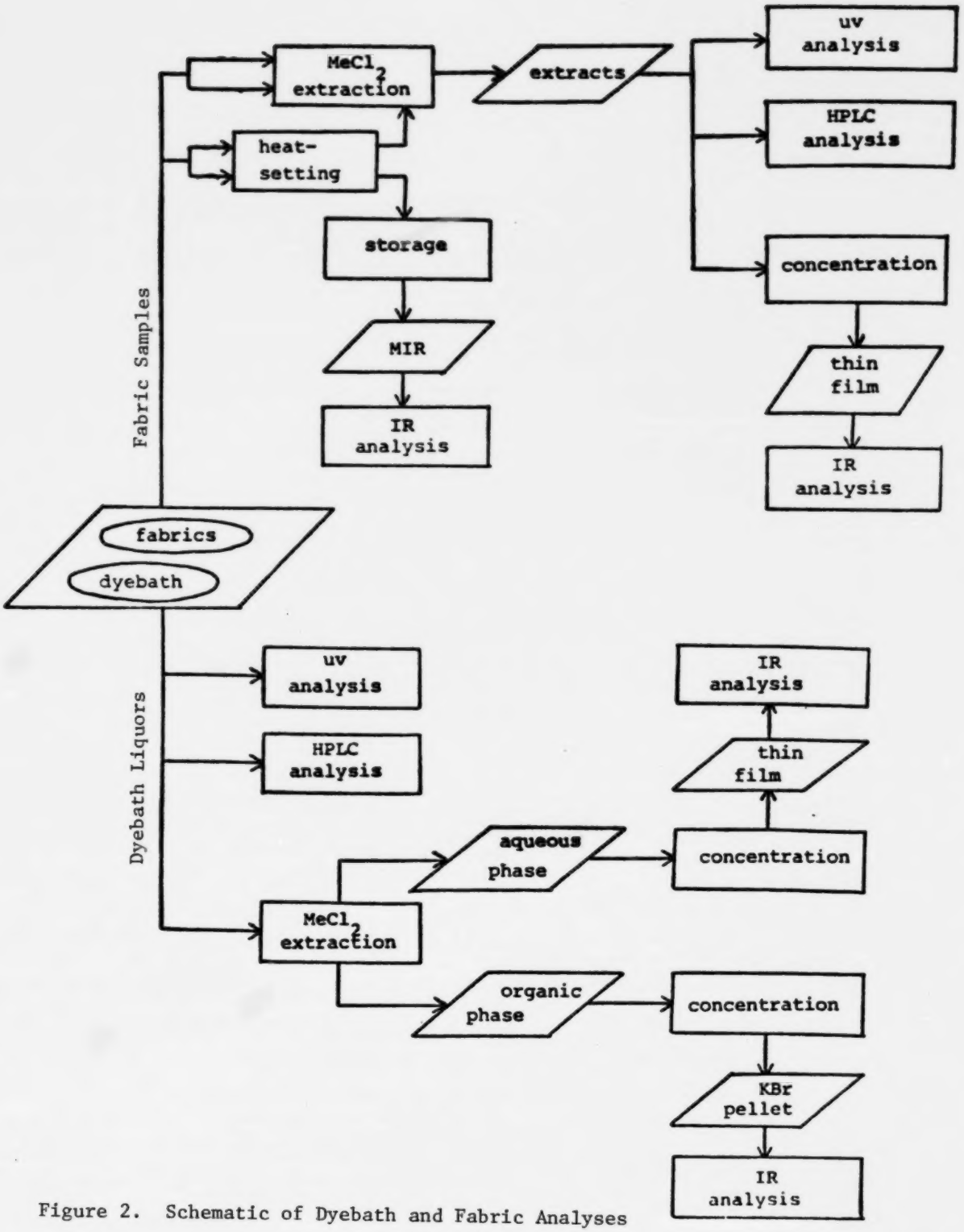


Figure 2. Schematic of Dyebath and Fabric Analyses

separate for 10 minutes. The organic methylene chloride layer was removed and placed in a 50 ml volumetric flask. Another 25 ml of methylene chloride was added to the separatory funnel and the extraction was repeated. The organic layer was removed and added to the 50 ml volumetric flask. The solution was made up to volume with methylene chloride and stored under refrigeration. The aqueous phase was collected and also stored under refrigeration.

The organic extract of the dyebath solution was concentrated by removal of the methylene chloride by evaporation. Twenty (20) ml of the methylene chloride extract was placed in a 100 ml round bottom flask. The solvent was removed under reduced pressure. Hot tap water (about 50°C) was used to warm the solution. The evaporation was continued until no additional solvent was removed. The residue remaining in the round bottom flask was recovered using a minimal amount of chloroform and stored in a small glass vial with a teflon-lined screw top. The aqueous phase of the dyebath was concentrated by evaporation in a hot water bath. Twenty (20) ml of the water solution was placed in a small glass vial and gently heated to dryness in a water bath on a hot plate. The residue was further dried in an oven at 110° for several hours prior to analysis.

Fabric Extraction

Dyed fabrics were extracted to determine the pick-up during dyeing and loss during heat setting. A piece of fabric to be extracted was first placed in 100 ml of methylene chloride and shaken for a few minutes. The process was repeated in another 100 ml of methylene chloride. The fabric was placed in a Soxhlet extraction tube and extracted with 250 ml of methylene chloride for a total of 8 dumps. The fabric was removed and air dried. The methylene

chloride extract was placed in a 500 ml volumetric flask. The two 100 ml initial methylene chloride rinse solutions were also added to the flask. To dissolve the small fraction of water present, 50 ml of acetone was added. The flask was then make up to volume with methylene chloride. The methylene chloride solutions were concentrated in the same manner as the dyebath solutions. A piece of undyed but scoured polyester fabric was also extracted as a blank following the same procedure. The extract obtained was measured by UV-visible spectrophotometry. The extract was concentrated and the residue identified by infrared analysis.

QUALITATIVE ANALYSIS PROCEDURES

An infrared spectrophotometer (Perkin Elmer) with computer (Data Station) interface was used for general qualitative analysis of all prepared samples.

IR-Data Station

The Perkin Elmer Model 3500 Data Station is an example of a microcomputer designed to be used with an analytical instrument such as the Perkin Elmer 281B spectrophotometer. The Data Station consists of 3 modules - a visual display unit, a data processing module and a keyboard. When the Data Station is connected to the infrared spectrophotometer, an integrated analytical data system results. The user sets the spectrophotometer controls and enters commands through the computer keyboard. The system's software consists of an operating program permanently stored in the data station and a variety of task programs stored on microfloppy disks which are placed in the disk drives for access. The software provides instructions for the Data Station. The data processing module interprets instructions and can command the spectrophoto-

meter, accept data from the spectrophotometer, store data in the working memory, perform spectral manipulations and finally make permanent storage of data on microfloppy disks. Commands as well as spectra are displayed on the visual display unit. The numeric display may be made into hard copy by the thermal printer. Spectra can be replotted on the spectrophotometer chart paper from the computer memory. Obey routines may be written and used with the Data Station which responds to the BASIC Language.

The solid state memory of the Data Station consists of 64 k bytes of read only memory (ROM) and random access memory (RAM). Data may be stored on soft sectored 5.25 inch microfloppy disks. A blank microfloppy disk consists of 624 sectors. Storage of a spectrum requires about 60 sectors and a peak table 8-12 sectors. One side of a microfloppy disk therefore can hold 8-10 full spectra or 80-100 peak tables of data. A spectrum is stored as a table of intensities for a given frequency of wavenumber data sampling, usually every wavenumber. A peak table is a table of the frequencies of the peaks in a spectrum and their corresponding transmittance values.

Application Programs

Three applications programs may be used with the Infrared Data Station. These are PECDS, SEARCH, and QUANT. PECDS performs routine processing of spectral data such as averaging, accumulating, smoothing, flattening, expanding and the taking of differences. PECDS is also used to store spectral data and retrieve it from a microfloppy disk. SEARCH is used in the identification of an unknown compound. The SEARCH applications program has the ability to compare spectra to a library of reference compounds and produce a list of the 15 best matches. The SEARCH library contains about 2000 compounds

obtained from a variety of sources. The user may add spectra to the library as desired. The SEARCH program also provides a list of the possible structural units (PSU) for a compound. These PSU indicate the functional groups present in a sample. The QUANT program is used to do quantitative analyses with the infrared spectrophotometer.

Characterization of Dyebath Components

Infrared spectra of all individual dyebath components and possible reaction products were prepared. Table V lists some of the spectra prepared and sampling technique employed. KBr pellets were prepared by drying the sample in an oven at 110°C, grinding the sample with a mortar and pestle and finally mixing it and pressing with spectral grade KBr. "Neat" (pure sample) thin films were prepared by placing a sample on a salt window (KBr or NaCl) and immediately covering it with another window. The "sandwich" of windows was placed in a demountable cell for spectrophotometer presentation. Thin films were prepared from solution by spotting a sample on a salt window and then allowing the solvent to evaporate before covering with the second salt window. Extremely volatile samples were placed in a sealed cell and observed neat. A library was prepared of those compounds which were not already in the SEARCH library of reference compounds. Those compounds placed in the library are marked in Table V with an asterik.

The spectra prepared were analyzed using the SEARCH applications program. Using the interpret command (INTRP), the data in the peak table for a spectrum was compared to about 850 possible structural units(PSU). Identified PSU were then displayed. A PSU reference manual was consulted to learn further information about the PSU indicated and any possible interferences. After

TABLE V. INFRARED SPECTRA PREPARED

Disperse Yellow 23	Sampling Technique
*Commercial presscake	KBr pellet
*Commercial presscake	thin film from acetone
*Commercial dye	KBr pellet
*Commercial dye--Soxhlet extracted dye portion	KBr pellet
*Commercial dye--Soxhlet extracted dye portion	thin film from acetone
*Commercial dye--Soxhlet extracted dispersing agent portion	KBr pellet
Commercial dye--TLC separated lower band	thin film from acetone
Commercial dye--TLC separated upper band	thin film from acetone
p-phenylazoaniline	KBr pellet
*p-phenylazoaniline	thin film from acetone
Trichlorobenzene Carrier	
*Commercial carrier	thin film, neat
*Commercial emulsifier	thin film, neat
Commercial emulsifier, methylene chloride extracted	thin film from methylene chloride
Pure 1,2,4--trichlorobenzene	thin film, neat
Pure 1,2,3--trichlorobenzene	thin film from carbon disulfide
Pure 1,3,5--trichlorobenzene	thin film from carbon disulfide

TABLE V. INFRARED SPECTRA PREPARED (CONTINUED)

Wetting Agent	Sampling Technique
*Commercial product	thin film, neat
Commercial product, methylene chloride extracted	thin film from methylene chloride
Fabric	
Polyester fabric as received from mill	MIR
Polyester fabric scoured	MIR
*Methylene chloride blank fabric extract	thin film from methylene chloride
Solvents	
Acetone	sealed cell, neat
Carbon disulfide	sealed cell, neat
Chloroform	sealed cell, neat
Methanol	sealed cell, neat
Methylene chloride	sealed cell, neat
Other	
* Acetic acid	thin film, neat
Tetrasodiumpyrophospate	KBr pellet
Silica gel from TLC chromatogram blank	KBr pellet

interpretation, the spectra were further identified by the SEARCH command which produces a list of the 15 best matches with the chemical compounds in list of the 15 best matches with the chemical compounds in the SEARCH library. The matches are ranked by the similarity of the match. The first score indicates how well the PSU match. The second score indicates how well the bands match. A score below 9-4 probably is not an actual match, a score above 9-4 has a high probability of being a match.

The MIXS command was used as an alternative to the SEARCH command. MIXS was used when a sample was known to be a mixture of compounds. With MIXS, a special algorithm is used for producing the list of 15 best matches. Scores are not penalized by the presence of a small fraction of extra peaks which do not match the reference compound. MIXS also allows the intensity of the peaks in the mixture to be lower.

The PECDS applications program was used to analyze the commercial products which were known to be mixtures of compounds. The difference command was used to subtract the spectrum of a known component of a mixture from the spectrum of the mixture. The difference function normalizes the intensities of the peaks of the compound to be subtracted so that the spectra are of the same relative concentration. The resulting difference spectra was expanded full scale using the ABEX command and then the spectra was identified by the SEARCH program. The commercial carrier and the commercial dye product were examined with this technique. The results were compared to the results of the chemical separation methods or the spectra of the known components.

ANALYSIS OF DYE WITH UV-VIS SPECTROPHOTOMETRY

Quantitative analytical procedure for the C.I. Disperse yellow 23 dye was established with a Beckman UV-VIS spectrometer. Calibrations were obtained with purified yellow 23 in both methylene chloride ($MeCl_2$) and acetone. The max. absorption peak for the yellow 23 was identified as: λ_{max} ($MeCl_2$) = 375 nm and λ_{max} (acetone) = 377 nm.

Calibration

Samples of pure yellow 23 were prepared from two different sources; a) a pure y-23 presscake obtained from commercial source was reprocessed, dissolved in acetone, filtered, dried over $NaHSO_3$ and recrystalized (C-C) and, b) a commercial y-23 formulation was extracted in Soxhlet with acetone, and the purified y-23 recrystalized (C-G). Both samples were used to prepare standard solutions in $MeCl_2$ for spectrophotometric calibration. The results are shown in Table VI. Data were then regressed by the Beer's Law:

$$c = (1/ab)\ A$$

where c = concentraton in mg/l

a = extinction coefficient

b = path length = 1 cm and,

A = absorbance

Regressions were obtained at both 365 and 375 nm for samples (C-C) and combined (C-C+C-G) as shown in Table VII. Results from two sets of samples, (C-C) and (C-G), are identical. The calibration equation for both sets combined at 375 nm was then selected to be used for this study. A calibration was also obtained in acetone using pure y-23 sample (C-G) and the results are shown in Table VIII. There are slight differences in the slopes of the calibrations in the two different solvents. Furthermore, the molar extinction

TABLE VI. CALIBRATION OF DYE (YELLOW 23, DISAZOPHENOL) IN $MeCl_2$

Soln.	Conc., mg/l.	Absorbance	
		365 nm	375 nm
C-C (reprocessed)[a]			
1	14.84	1.636	1.736
2	11.87	1.337	1.406
3	8.31	.930	.982
4	5.94	.658	.689
5	2.97	.339	.352
6	1.19	.136	.142
7	.83	.094	.099
8	.59	.078	.082
C-G (Soxhlet)[b]			
9	11.97	1.300	1.386
10	8.38	.918	.967
11	5.99	.687	.703
12	2.99	.350	.368
13	1.20	.128	.135

[a] dissolved in acetone, dried over $NaHSO_3$, filtered and recrystalized.

[b] extracted in soxhlet with benzene.

TABLE VII. REGRESSION OF THE CALIBRATION RESULTS FOR YELLOW 23 IN $MeCl_2$

C-C (8 pts.)

@ 375 nm.

$C = 8.50A$ mg/l

$\frac{1}{ab} = 8.50 \pm .05$

$\gamma = .9999$

@ 365 n.m.

$C = 8.98A$ mg/l

$\frac{1}{ab} = 8.98 \pm .06, \gamma = .9999$

C-C + C-G (13 pts.)

@ 375 nm.

$C = 8.56A$ mg/l

$\frac{1}{ab} = 8.56 \pm 0.06, \gamma = .9997$

$a = 116.82$ l/g cm

ε = molar extinction coeff.

$= 35,198$ l/mole-cm

@ 365 nm.

$C = 9.02A$ mg/l

$\frac{1}{ab} = 9.02 \pm .08, \gamma = .9997$

TABLE VIII. CALIBRATION OF DYE (Y-23) IN ACETONE

Soln.	Conc. $^{mg}/_{l}$	Absorbance 375 nm.	Absorbance 377 nm.
1	17.00	1.940	1.970
2	13.60	1.507	1.513
3	10.20	1.122	1.132
4	8.50	.950	.957
5	6.80	.774	.778
6	3.40	.394	.397
7	1.70	.235	.237
8	0.85	.104	.105

@ 375 nm.

$C = 8.88A \ ^{mg}/_{l}$

$\frac{1}{ab} = 8.88 \pm .14$

$\gamma = .9994$

$a = 112.61 \ ^{l}/_{g\text{-}cm}$

and

ε = molar extinction coeff.

$= 33{,}598 \ ^{l}/_{mole\text{-}cm}$

coefficients are 35,198 and 33, 598 1/mole-cm, respectively in $MeCl_2$ and in acetone. These values are within the literature values of 25,000-35,000, 1/mole-cm.

Characterization of Commercial Yellow 23 Formulation

Yellow 23 content in the commercial dye formulation was determined via 1) gravimetric determination in the Soxhlet extraction and 2) spectrophotometric determination of yellow 23 in dyebath solutions containing commercial dye, carrier and other additives. Results of both determinations are shown in Table IX. The spectrophotometric method was carried out in the following manner: Two dyebaths, with different composition of carrier (Table X) were prepared at room temperature. Each bath was divided into two portions (Soln A and Soln B). Solution B's were then proceeded with mock dyeing (without fabrics), cooled to room temperature and pH adjusted. A stock solution was prepared each from Soln. 1-A and Soln. 1-B, by diluting the aqueous solutions with acetone at 1 to 4 ratio. A series of standard solutions were prepared from these two stock solutions, and absorbance at 375 nm obtained. The slopes from the regression equations of both solutions at 375 nm are essentially the same, suggesting that the dyeing procedure (heating and all) does not interfere with the spectrophotometric measurement at 375 nm. In order to check if there was any interference on spectrophotometric measurement of y-23, due to variation of dyebath composition, the #2 dyebath solution was prepared in identical conditions as the #1 bath. Both Soln. 2-A and Soln. 2-B were analyzed for dye content, and the results are also shown in Table X. The difference between the measured and calculated concentrations is less than 0.5% in both cases.

TABLE IX. ANALYSIS OF Y-23 CONTENT IN COMMERICAL FORMULATION

I. Extraction (Soxhlet) Method*

Sample	Initial wt. Comm. dye, g.	Wt. lost (extracted), g.	% Y-23 in Comm. dye
1	0.59138	0.24068	40.70
2	0.21444	0.08874	40.92
3	0.28610	0.11570	40.44
4	0.61471	0.24931	40.56
5	0.42058	0.17008	40.44
6	0.45116	0.18136	40.20
			Avg. 40.54± .25

*solvent - acetone

II. Spectrophotometric Method

Sample	Conc. mg/l Comm. Dye	A @ 375 nm	Conc. mg/l Y-23	% Y-23 in Comm. Dye
Soln 1-A*				
1	34.90	1.597	14.36	41.1
2	27.92	1.275	11.46	41.1
3	20.94	.948	8.52	40.7
4	17.45	.810	7.28	41.7
5	13.96	.633	5.69	40.8
6	6.98	.324	2.91	41.7
7	3.49	.161	1.45	41.5
8	1.75	.084	.76	43.2
Soln 1-B**				
9	35.15	1.580	14.20	40.4
10	28.12	1.269	11.41	40.6
11	21.09	.940	8.45	40.1
12	17.57	.767	6.90	39.2
13	14.06	.624	5.61	39.9
14	7.02	.316	2.84	40.4
15	3.52	.167	1.50	42.7
16	1.76	.075	.67	38.3
				Avg. 40.8± 1.2

*prepared at room temp.
**mock dyeing

TABLE X. DYEBATH COMPOSITION OF TRIAL DYEINGS

Bath vol.: 1.0 liter (40: 1)

#1

Dyestaff (yellow 23)	:	175.73 mg
Carrier (TCB)	:	3.50443 g
Wetting agent	:	100.01 mg

#2

Dyestaff (yellow 23)	:	176.43 mg
Carrier (TCB)	:	7.02240 g
Wetting agent	:	101.43 mg

bath #2

dye conc. = 176.43 mg/l

analyzed conc. :

Soln. 2-A = 177.30 mg/l (-0.5%)

Soln. 2-B = 175.88 mg/l (0.3%)

A blank dyeing (dyebath with all additives but dye) was also carried out and the dyebath liquor after dyeing was also analyzed with spectrometer for any absorption at 375 nm. Results indicated the absorbance of blank bath at 375 nm was negligible.

Procedure for Dyebath Liquid Samples

Aqueous dyebath samples from screening were diluted with at least an equal volume of acetone. Absorbances at 375 nm of these acetone-diluted sample were then obtained and yellow 23 concentration in these samples were calculated with the established calibration in acetone (Table VIII).

Procedure for Fabric Sample

Extraction Method --

Dyed fabric samples (those directly from dye bath and those scoured and cured) were each placed in a Soxhlet extraction tube and extracted with $MeCl_2$. Each of the $MeCl_2$ extract was then analyzed for dye and carrier contents. A blank extraction was also carried out with undyed, pre-scoured fabric. Extractions were continued for eight (8) turnovers, as it was predetermined that 6-7 turnovers were required to remove all chemical (dye and carrier) pick-ups from the fabric.

Dye analysis of the extract was done without any dilution or alteration of the sample. Calibration of y-23 in $MeCl_2$ was used. The extract from the blank extraction gave slight absorbance at 375 nm, and this absorbance was corrected for all samples before concentration was calculated. The compound in the blank extracts, which gave slight absorbance at 375 nm. was identified

with IR/computer search to be a low molecular weight (oligomer) polyester, obviously from the fabric.

Calorimetric Method --

A rapid and non-destructive procedure to determine dye content on the dyed fabrics was also established, using a Hunter Calorimeter. A total of 106 dyed fabric samples were prepared through 28 different dyebaths, as shown in Table XI. Thirteen (13) of the dyebaths were prepared with various concentration of biphenyl carrier, and the other fifteen (15) baths with trichlorobenzene carrier. The colorimetric values [7-9], L, a, b, of all these fabric samples were determined with a Hunter Colorimeter. A standard yellow color tile was used as a reference. The total color difference, ΔE, was then calculated as the root-square mean of differences in colorimetric values;

$$\Delta E = [(\Delta L)^2+(\Delta a)^2+(\Delta b)^2]^{1/2}$$

where ΔL, Δa and Δb are the differences in L,a,b, values between the dyed fabric sample and the standard yellow tile.

Actual dye contents of these dyed samples were then determined, using a combined wet chemistry and spectrophotometric technique.[10] A 1 x 1 inch square sample was cut from each dyed fabric sample. The square was weighed and placed in a 50 ml. volumetric flask. Approximately 3 ml. of chlorobenzene (reagent grade) and 3 g. of phenol (reagent grade) were added to the 50 ml. flask. The flask was heated in a 90-100°C oil bath until the sample dissolved. The contents of the flask were cooled and diluted to volume with acetone (spectro-grade). The polyester precipitates when the acetone is added and the solution becomes clear. The clear dye solution was removed with a pipette.

TABLE XI. DYEBATH COMPOSITIONS

Bath No.	Carrier	Weight (g)
BP1	BP-90	4.472
BP2	BP-90	3.480
BP3	BP-90	6.108
BP4	BP-90	8.446
BP5	BP-90	3.103
BP6	None	0
BP7	BP-10	35.014
BP8	BP-90	2.758
BP9	BP-10	3.050
BP11	BP-10	3.010
BP12	BP-10	3.050
BP13	BP-10	1.502
BP14	BP-10	1.712
TCB2	TCB	2.591
TCB3	TCB	5.549
TCBS-1	TCB	3.524
TCBS-2	TCB	3.487
TCBS-3	TCB	3.499
TCBG-3	TCB	3.501
TCBG-4	TCB	3.496
TCBG-5	TCB	3.501
TCBG-6	TCB	3.502
TCBG-7	TCB	3.498
TCBG-8	TCB	3.501
TCBG-9	TCB	3.498
TCBG-10	TCB	3.502
TCBG-11	TCB	3.505
TCBG-12	TCB	3.502

Dye concentration of the solution was determined with the spectrophotometric procedure, described earlier, using the established calibration in acetone; Dye content on the original fabric sample was then calculated as mg/g fabric.

Analyzed dye contents of these fabric samples, together with the determined colorimetric values, L, a, b, and total color difference, ΔE, were tabulated in Table XII. A linear relationship between the dye content, [D], and total color difference, ΔE, was observed for these fabric samples. A regressed linear equation was obtained as:

$$[D]\ (mg/g) = 0.654 \times \Delta E - 7.41$$

This calibration equation was used in all subsequent colorimetric determinations of fabric sample dyed with yellow 23.

ATTEMPTED QUANTITATIVE ANALYSIS OF CARRIER WITH UV-VIS

Attempt was initially made to establish a quantitative analytical procedure for trichlorobenzene(TCB) carrier, using an UV spectrophotometer. Since TCB carrier contains all three isomers of TCB, it was necessary to establish calibrations of individual isomers at three different wavelengths, before solution of individual concentration in the mixture can be obtained. 1,2,4 TCB has a dominate absorption (forbidden band) at 287 nm, whereas, all three isomers absorb in the region of 250-275 nm. Calibrations for each of the isomers were obtained individually at 287, 273, 265 and 260 nm (Table XIII), using pure chemicals. With calibration constant, a, listed in Table XIII, following three equations can be established at 287, 273 and 265 nm;

$$A_{287} = 2.837\ x + .216y + .230z$$

$$A_{273} = 1.700\ x + .953y + 1.062z$$

TABLE XII. DYE CONTENTS AND COLORIMETRIC VALUES OF THE DYED FABRICS

[D] , MG/G	L	A	B	Δ E
0.0	78.2	-2.5	21.5	0.00
0.0	66.3	-3.1	-5.1	29.15
5.1	60.2	2.7	28.0	19.83
5.4	60.2	2.3	28.0	19.73
5.6	60.6	1.7	27.6	19.09
5.3	60.5	2.0	27.7	19.29
5.0	60.6	1.3	27.7	19.04
4.8	60.9	1.5	27.6	18.77
4.7	60.8	1.8	27.6	18.93
5.1	60.7	1.8	27.7	19.06
2.7	63.4	-3.0	23.4	14.93
2.7	63.4	-2.6	23.8	14.98
2.7	63.4	-3.1	23.4	14.93
2.9	63.3	-3.0	23.1	14.99
4.0	61.9	-0.2	25.9	17.04
4.2	61.9	0.1	26.4	17.22
4.1	62.4	-0.4	26.0	16.56
3.8	62.3	-0.5	26.0	16.65
3.7	61.8	-0.7	26.1	17.13
3.5	62.2	-0.6	26.4	16.84
3.8	61.6	-0.5	26.1	17.34
3.9	61.8	-0.3	26.5	17.29
6.4	58.8	2.5	27.1	20.80
6.4	59.0	2.9	27.6	20.86
6.3	58.5	3.2	27.7	21.42
6.4	59.1	2.9	27.5	20.74
4.0	62.2	1.0	26.4	17.10
4.0	61.6	0.6	25.9	17.45
3.3	62.5	-0.4	25.9	16.44
3.5	62.6	-1.0	25.7	16.22
4.8	61.1	0.8	27.0	18.26
5.1	60.7	2.0	27.8	19.14
4.8	61.2	1.0	27.3	18.30
5.0	60.9	1.0	26.9	18.46
6.9	58.7	4.4	29.4	22.14
7.8	57.7	6.4	30.0	23.91
7.6	57.8	6.5	30.2	23.93
8.1	57.7	6.0	29.8	23.69
8.3	57.6	6.3	29.9	23.92
8.2	58.2	5.0	29.8	22.92
7.6	58.1	5.6	29.7	23.17
7.2	58.4	4.8	29.6	22.60
6.8	58.0	6.6	30.1	23.77
7.1	57.9	7.0	30.4	24.12
6.5	57.7	6.3	30.0	23.87
8.2	57.7	6.6	30.2	24.06
6.9	57.7	5.4	29.1	23.25
7.6	58.2	5.8	29.6	23.12
6.9	58.5	6.3	30.0	23.19
7.8	58.0	5.9	29.7	23.36
8.9	57.2	6.8	29.9	24.46

TABLE XII. DYE CONTENTS AND COLORIMETRIC VALUES OF THE DYED FABRICS (CONT.)

[D], MG/G	L	A	B	ΔE
7.2	57.4	6.0	29.6	23.89
7.3	58.2	5.1	29.4	22.81
5.9	59.0	3.6	28.8	21.43
7.1	58.8	5.7	29.5	22.53
7.8	58.2	5.9	29.5	23.12
7.8	57.5	7.9	30.2	24.75
8.4	58.4	6.4	29.8	23.24
7.1	59.3	4.7	29.2	21.64
6.4	59.4	5.1	29.4	21.76
6.2	59.3	5.6	29.6	22.10
6.0	59.0	5.7	29.5	22.36
7.2	58.6	5.3	29.4	22.53
6.8	58.7	5.1	29.4	22.37
6.8	59.5	4.9	29.6	21.68
6.5	59.6	4.7	29.5	21.49
7.2	58.5	6.3	29.8	23.12
7.7	58.5	5.8	29.6	22.86
7.5	58.7	5.4	29.5	22.51
7.1	58.7	4.4	28.7	21.90
8.4	57.7	6.9	29.8	24.03
6.6	58.3	4.6	29.0	22.42
6.9	58.4	5.0	29.1	22.50
7.7	58.2	6.1	29.4	23.16
7.7	58.1	6.2	29.6	23.35
7.7	58.4	5.3	29.4	22.70
6.9	58.3	5.1	29.1	22.62
8.0	58.0	5.8	29.2	23.16
8.1	58.3	6.1	29.6	23.14
8.2	58.8	5.2	29.1	22.21
7.0	58.6	5.0	29.1	22.32
7.3	58.1	5.4	29.0	22.86
7.2	58.5	5.5	29.2	22.61
8.5	58.1	6.5	29.7	23.50
8.3	58.0	6.0	29.3	23.26
8.8	57.9	7.2	29.9	24.02
8.2	58.6	5.0	28.9	22.25
7.9	58.6	5.8	29.5	22.74
7.8	58.3	6.4	29.6	23.26
7.6	58.5	4.8	28.9	22.27
7.5	58.1	6.0	29.3	23.18
8.4	58.4	5.5	29.1	22.67
8.0	57.9	6.2	29.6	23.52
6.3	59.2	4.7	29.4	21.00
7.3	57.8	5.6	29.5	23.36
7.9	57.7	5.7	28.8	23.25
7.4	58.3	5.3	29.9	22.97
7.6	58.3	5.0	29.3	22.65
8.3	58.1	7.0	30.3	23.91
7.9	57.6	5.9	29.1	23.51
7.8	58.2	4.7	28.6	22.41

TABLE XII. DYE CONTENTS AND COLORIMETRIC VALUES OF THE DYED FABRICS (CONT.)

[D], MG/G	L	A	B	ΔE
6.4	58.8	3.9	29.1	21.80
6.9	58.6	6.0	29.8	22.92
7.5	58.5	4.6	29.2	22.31
6.7	57.3	5.5	29.5	23.77
7.7	57.3	6.3	29.5	24.05
7.0	58.0	5.0	28.9	22.78
7.7	57.9	6.1	29.7	23.52

TABLE XIII. SPECTROPHOTOMETRIC CALIBRATION OF TCB ISOMERS ($MeCl_2$)

1,2,4 TCB	a 1/g-cm	ε 1/mole-cm
287 nm	2.837±.023	514.9
273 nm	1.700±.019	308.6
265 nm	1.094±.018	198.6
260 nm	.729±.016	132.3
1,2,3		
287 nm	.216±.004	39.2
273 nm	.953±.008	173.0
265 nm	.711±.007	129.0
260 nm	.497±.005	89.3
1,3,5 TCB		
287 nm	.230±.003	41.7
273 nm	1.062±.004	192.8
265 nm	.778±.012	141.2
260 nm	.481±.009	87.3

$$A_{265} = 1.094\,x + .711y + .778z$$

Where, A is absorbance at each of the three wave lengths, x, y, z are concentrations (in g/l) of 1,2,4, 1,2,3 and 1,3,5 TCB isomers.

Simultaneous solution of the above three equations would allow determination of individual isomer concentrations in the mixture. Two solutions containing known amount of each isomers were prepared to test the accuracy of this procedure. The results, shown in Table XIV, indicated that the procedure can not be used for quantitative determinations of 1,2,3 and 1,3,5 isomers. This was due mainly to the significantly much larger extinction coefficient of the 1,2,4 isomer, as compared to those of the other isomers. It was found subsequently that both the dye (yellow 23) and emulsifier also absorb in the region of 250-270 nm. This would further interfere with the spectrophotometric determination. Therefore, the procedure was abandoned.

ANALYSIS OF CARRIERS WITH HPLC

The following instrumentation was used in the development of procedures for separation and identification of trichlorobenzene(TCB) and biphenyl(BP) carriers, in both the dyebath samples and extracts of fabrics;

(1) Perkin-Elmer Series 3B Liquid Chromatograph,

(2) Perkin-Elmer LC-75 Spectophotometric detector and LC-75 Autocontrol,

(3) Reverse phase C_8 column of length 25 cm. and ID 0.46 cm having a particle size of 10

(4) Rheodyne Model 7125 Syringe Loading Sample Injector.

TABLE XIV. ACCURACY OF SPECTOPHOTOMETRIC DETERMINATIONS OF TCB ISOMERS

$$A287 = 2.837\,X + .216y + .230z$$

$$A273 = 1.700\,X + .953y + 1.062z$$

$$A265 = 1.094\,X + .711y + .778z$$

$$X = \frac{\begin{vmatrix} A287 & .216 & .230 \\ A273 & .953 & 1.062 \\ A265 & .711 & .778 \end{vmatrix}}{M} = 1,2,4\ TCB$$

$$Y = \frac{\begin{vmatrix} 2.837 & A287 & .230 \\ 1.700 & A273 & 1.062 \\ 1.094 & A265 & .778 \end{vmatrix}}{M} = 1,2,3\ TCB$$

$$Z = \frac{\begin{vmatrix} 2.837 & .216 & A287 \\ 1.700 & .953 & A273 \\ 1.094 & .711 & A215 \end{vmatrix}}{M} = 1,3,5\ TCB$$

$$M = \begin{vmatrix} 2.837 & .216 & .230 \\ 1.700 & .953 & 1.062 \\ 1.094 & .711 & .778 \end{vmatrix} = .03524$$

		conc. g/l.	calc. conc. g/l.
I.	X	.41613	.43658
	Y	.05423	-.75254
	Z	.06779	.80117
II.	X	.20807	.22165
	Y	.02712	-.45076
	Z	.03340	.46373

Trichlorobenzene Carrier

Characterization of Commercial Formulation --

TCB contents in the commercial formulation were analyzed first using gas chromatography. After retention times of the isomers were identified with pure individual isomers, a commercial carrier sample was prepared in $MeCl_2$ and separated in GC. The relative peak heights for the three isomers were calculated as the relative concentrations of these isomers. The result is shown in Table XV. The content of total TCB in the commercial formulation is 93%, based on information obtained from the supplier. And with the relative composition of the three isomers determined with GC, the 1,2,4 TCB content in the formulation was calculated as 69.8%.

As mentioned earlier, all three isomers of TCB give absorbance in the UV region, 260 290 nm. However, 1,2,4 TCB (predominant component of the carrier) has a strong absorption at 287 nm where the interferences from the other two isomers and the emulisifier are small. 1,2,4 TCB can be analyzed at 287 nm. By ignoring the interferences, an estimated error of 3% (if dye is not present) will be in the results of samples, such as commercial carrier. This method of analyzing 1,2,4 TCB is not quantitative; however, it is a valuable means of cross-checking the results from other determinations. 1,2,4 TCB content in the commercial formulation was analyzed with this method as 71.5% (Table XV). This is in agreement with the result obtained with GC.

Calibration of TCB in Organic Solvent

A mixture of the 3 isomers of TCB, containing each isomer approximately in the ratio 1:1:1 was made in acetonitrile. The attempt to separate the isomers using methanol as the stronger solvent, (solvent A) and water as the

Table XV. 1,2,4 TCB Content in Commercial Carrier Formulation

I. GC Analysis of relative composition of isomers*

Isomer	Relative composition, %	% of formulation**
1,2,4	75.0	69.8
1,2,3	10.5	9.8
1,3,5	14.5	13.4

II. Spectrophotometric Method (in $MeCl_2$)

Sample	Carrier Conc. mg/l.	1,2,4* Analyzed mg/l.	% 1,2,4 in Formulation
1	394.4	280.1	71.2
2	597.8	428.6	71.7

*@287 nm.

*commercial formulation in $MeCl_2$
**commercial formulation: 93% TCB, 7% emulsifier

other solvent, (solvent B) in different proportions as the mobile phase was not successful. It was decided to try acetonitrile as Solvent A and water as Solvent B. A solvent flow program for best separation was obtained for the microprocessor-controlled dual-pump unit with variable wavelength UV detector. The program includes flow rate of 1.7 ml/min and varying concentration of the two solvents in the mobile phase. The UV detector was set at 278 nm.

The retention times for the 3 isomers in a mixture containing approximately equal quantities of the 3 isomers were around 15.9 min for 1,2,3 - TCB, 17.4 min for 1,2,4 - TCB and 18.7 for 1,3,5 - TCB. These values could vary by a few 10ths of a minute.

A mixture of the 3 isomers of TCB in the ratio 11:75:15 for 1,2,3, 1,2,4 and 1,3,5 - TCB was then taken. This ratio was close to the 10:70:13 ratio for the 3 isomers in the TCB carrier. The above solvent programs were used at 278 nm, 1,2,3 - TCB appeared as a very small step around 16.4 min, which was immediately followed by the large peak of 1,2,4 - TCB. A fairly well resolved peak of 1,3,5 - TCB followed around 19.2 min.

Using the same experimental set-up and conditions, a calibration was obtained for the response of the detector at 278 nm, for different concentrations of 1,2,4 TCB in acetonitrile. The concentrations used varied from 720 mg/l down to 45 mg/l. The results are shown in Table XVI. Regression through the origin of the straight line obtained gave the following equation:

$$\text{Conc. (mg/l)} = 2.04 \times \text{peak height},$$

where peak height is in cm x attenuation. The volume injected each time was 8 ml.

Table XVI. Calibration of 1,2,4 TCB in Acetonitrile

Peak Ht. = Peak Height (in cm) * Attn
Conc. (mg/l) = B x(Peak Ht.)

N=8 Slope = 2.040 (SD) Slope = 0.035 Doeff = 0.99927

X (Peak Ht.)	Y (Conc.)	BX	Y - BX	% Diff.
361.600	719.730	737.556	%-17.826	-2.48
257.600	539.800	525.427	14.373	2.66
193.600	404.850	394.866	9.964	2.46
175.200	359.870	357.356	2.514	0.70
86.800	179.940	177.046	2.894	1.61
75.200	149.700	153.386	-3.686	-2.46
40.500	89.970	82.608	7.362	8.18
19.500	44.990	39.774	5.216	11.59

When a solution of known concentration of TCB carrier in acetonitrile was analyzed using the above solvent program at 278 nm, only one peak, which was less sharp than the pure 1,2,4 - TCB peak was obtained at a retention time of around 17.5 min. in the chromatogram. From the peak height and the calibration for 1,2,4 - TCB concentrations obtained earlier, the percentage of 1,2,4 - TCB in the TCB carrier was obtained as 71.0%. This is in agreement with previous results from UV and GC determinations (Table XV).

Determination of TCB in Aqueous Solution

When an aqueous solution of TCB carrier (140.3 mg/l) was analyzed using the above HPLC program at 278 nm, only about 40% of the 1,2,4 TCB present was detected. This was due to interference from the emulsifier present. Treating the solution with 1) NaCl and 2) NaOH, in an attempt to break up emulsion, did not result in any improvement of the amount of 1,2,4 TCB detected.

A Sep pak C_{18} cartridge was then used for preparation of the aqueous solution of TCB carrier, for injection into the HPLC. The cartridge was welted first with about 3 ml of acetonitrile(ACN) and then with about 5 ml water, before using it in the preparation of the sample. 25 ml of an aqueous solution of TCB carrier (140.3 mg/l) was passed slowly through the C_{18} cartridge. The cartridge was then washed with about 9 ml of ACN and the solution was made up to 10 ml with ACN. 8 ml of this solution was then injected into the HPLC. Two separately prepared samples of the aqueous solution of TCB carrier(140.3 mg/l) gave 74% and 61% as the amount of 1,2,4 - TCB present that could be detected.

It was decided to add a large excess of emulsifier AB-160, when preparing the aqueous solutions of TCB carrier, to check if this would interfer with the procedure. Small amounts of dye (disperse yellow 23) was also added in addition to the large excess of AB-160. This was to see if the presence of dye in the aqueous solutions would change the detection of 1,2,4 TCB. The results obtained for the aqueous solutions of TCB carrier of varying concentrations containing AB-160 or AB-160 and disperse yellow 23 are given in Table XVII. The results are consistent with the amount of 1,2,4 TCB present that could be detected being 69.4 with a standard derivation of 2.75. This procedure was followed in all TCB determinations of dyebath samples.

Determination of TCB on Fabric Samples --

Dyed fabric samples were first extracted with $McCl_2$ using procedures described earlier. The $MeCl_2$ extracts were analyzed for 1,2,4 TCB directly, using the HPLC program described above.

Biphenyl Carrier

The commercial biphenyl(BP) carrier used in this study, Carolid RNO, contains significant amounts of organic solvent, o-chlorotoluene(OCT) (Table III). An HPLC Program was first developed to separate BP and OCT in order that quantitative determination of BP and possibly, OCT, can be obtained.

The HPLC in Solvent Program mode was used for analysis of the BP carrier, Carolid RNO, in ACN solution. In this analysis the stronger solvent, A, was CH_3OH and the other solvent, B, was water.

With the above program, BP was analyzed at 246.5 nm, whereas OCT was detected at 272 nm. Autocontrol on the UV detector, LC-75, was used in the

Table XVII. Analysis of 1,2,4 TCB in Aqueous Carrier Solutions

	Sample Contents*				
	TCB Carrier mg/l	1,2,4 - TCB mg/l	AB-160 mg/l	1,2,4 - TCB** Detected Conc./mgl^{-1}	% of 1,2,4 - TCB Detected
	161.9	114.9	255	77.6	67.5
*	161.9	114.9	255	78.1	68.0
	151.1	107.3	255	75.4	70.3
	129.5	92.0	306	67.8	73.8
	107.9	76.6	233	54.2	70.9
	86.3	61.3	306	41.5	67.7
*	86.3	61.3	306	40.4	65.9
	75.6	53.6	309	36.3	67.7
*	75.6	53.6	309	38.8	72.5
*	64.8	46.0	306	33.7	73.2
	54.0	38.3	311	25.3	66.3
*	54.0	38.3	311	27.7	72.4
	32.4	23.0	510	15.9	69.0
*	32.4	23.0	510	15.2	66.3
					$\bar{n}$ 69.4
					S.D. 2.75

* The solution also contained 9.76 mg/l of dye, disperse yellow 23.

**Calculated with the calibration (Table XVI).

wavelength change mode: λ_1 = 246.5 nm and T_1 = 12.5 min.; λ_2 = 272 nm and T_2 = 18.0 min.

Calibration of BP and OCT in Acetonitrile --

A series of solutions containing known concentrations of pure BP in ACN were prepared. Quantitative calibration of BP was established using these known samples and the above HPLC Program. The results are shown in Table XVIII. Linear regression of BP concentration vs peak height was carried out, and a calibration constant was obtained, as shown in Table XVIII.

Similarly, calibration of OCT was also established and the results tabulated in Table XIX.

Characterization of Commercial Formulation --

A series of solutions containing known quantity of carrier, Carolid RNO, in ACN were then prepared. BP and OCT concentrations in these samples were determined, using the above HPLC program and calibrations. The results are shown in Table XX.

Determination of BP and OCT in Aqueous Solutions --

In order to analyse aqueous solutions of dye carrier, samples were prepared for injection into the HPLC using a Sep-pak C_{18} cartridge. The use of this cartridge made it possible to transfer the dye carrier from aqueous solution to an organic solution, at the same time concentrating the solution if necessary. The procedure was as follows: A Sep-pak C_{18} cartridge was first welted with about 5 ml ACN by forcing the ACN through the

TABLE XVIII. Calibration of Biphenyl (BP) at 246.5 nm*

BP Conc. (Mg/L) = B*(PK × Attn)

N = 7 Slope (B) = 0.258 $(SD)_{slope}$ = 0.008 Coeff = 0.99797

Conc Mg/l	Pk Cm**	Attn***	Calc	Conc - Calc	% Diff
17.00	17.4	4	17.97	-0.97	-5.72
33.90	17.2	8	35.53	-1.63	-4.81
50.90	6.2	32	51.23	-0.33	-0.65
85.40	10.6	32	87.59	-2.19	-2.56
134.90	8.6	64	142.12	-7.22	-5.35
169.60	10.3	64	170.21	-0.61	-0.36
186.40	10.8	64	178.48	7.92	4.25

*Solvent = acetonitrile
**PK = Peak height, Cm., values are average of three runs per sample.
***Attn = Attenuation

TABLE XIX. Calibration of o-chlorotoluene (OCT) at 272 nm*

OCT CONC. (mg/l) = B X (PK X ATTN)

N = 6 Slope (B) = 2.241 (SD) Slope = 0.078 Coeff = .998848

Conc, mg/l.	Pk, cm**	ATTN	Calc	Conc-Calc	% 5
43.1	5.4	4	48.40	-5.30	-12.29
64.7	7.1	4	63.64	1.06	1.64
107.8	6.6	8	118.31	-10.51	-9.75
150.9	8.3	8	148.78	2.12	1.40
215.5	12.0	8	215.11	0.39	0.18
258.6	7.1	16	254.54	4.06	1.57

*Solvent - acetonitrile
**Pk = Peak Height, cm, values are average of three runs per sample.
***ATTN = altenuation

TABLE XX. HPLC ANALYSIS OF BIPHENYL (BP) AND O-CHLOROTOLUENE (OCT) CONTENTS IN COMMERCIAL FORMULATION

Carrier Conc.*,	BP Detected		OCT Detected	
mg/l	Conc., mg/l**	% of Carrier	CMC., mg/l**	% of Carrier
81.6	21.2	25.9		
101.9	26.6	26.1	46.0	45.2
203.9	52.1	25.6	106.0	52.0
306.1	79.0	25.8	132.2	43.2
	Average	25.8		46.8

* in actonitrile

** values are average of at least three (3) runs

cartridge using a 30 ml syringe. It was then washed with about 10 ml of distilled water. 25 ml of the aqueous solution of the sample was then passed through the Sep-pak C_{18} cartridge. The solution eluting from the cartridge was discarded. About 4 ml ACN was then pased through this Sep-pak C_{18} cartridge and collected in a 5 ml volumetric flask. The solution was made up to 5 ml with ACN. The concentration of this solution is five times that of the original solution, assuming that all the dye carrier in the aqueous solution was transferred into the organic solution. This increase in concentration is necessary when analysing samples containing small concentrations of OCT, as the response of the HPLC system to OCT was low.

Aqueous solutions of the samples containing Carolid RNO were prepared for HPLC analysis using the Sep-pack C_{18} cartridges as described above. 8 ml of a prepared sample were injected into the HPLC and the chromatogram was obtained by using the experimental procedure described earlier. Measuring the peak heights from the chromatograms and using the calibration curves obtained, the concentrations of BP and OCT in the samples were determined. The results are shown in Table XXI. Linear regression of results in Table XXI gave calibration equations for BP and OCT in aqueous solutions. These calibrations were used in all determination of BP and OCT in dyebath and other aqueous sample containing BP and OCT.

Determination of BP and OCT on Fabric Samples --

Similar to the procedure used for determination of TCB on fabric samples, above described HPLC program were used to determine BP and OCT contents in the $MeCl_2$ extracts of dyed fabrics.

TABLE XXI. DETERMINATION OF BP AND OCT IN AQUEOUS SOLUTION USING SEP PAK

Carrier[1], mg/l	BP[2], mg/l	BP pk x attn[3]	OCT[2] mg/l	OCT pk x attn
40.8	10.6	98.1	19.1	29.3
81.6	21.2	188.3	38.2	50.1
122.4	31.8	357.4	57.3	94.9
163.2	42.4	442.7	76.4	125.9

Regressed calibrations:

BP conc. ($^{mg}/l$) = 0.0954 x (pk x attn)

OCT conc. ($^{mg}/l$) = 0.6211 x (pk x attn)

[1] Carolid RNO

[2] BP content = 25.8%, OCT content = 46.8% of carrier

[3] Peak height x attennation, at 246.5 nm, recoder at 10 mv.

[4] Peak height x attennation, at 272 nm, recoder at 1 mv.

SECTION 5

RESULTS AND DISCUSSION

SCREENING STUDIES

Three (3) screening studies were carried out on the selected commercial carrier systems. Those were trichlorobenzene (TCB), biphenyl (BP) and o-phenylphenol (OPP) carrier systems. Dyeing procedure (Table IV) and analytical sampling procedure (Figure 1), previously described, were followed. The dyebath composition for each of the screening studies is shown in Tables XXII, XXIII and XXIV. Results of bioassay and chemical analyses are discussed herein.

Bioassay Results

Results of bioassays; 1) Ames Salmonella/Microsome Mutagenesis Assay, 2) acute IN VIVO Mouse Quantal Assay and, 3) Daphnia magna Assay, on each of the three screening studies were obtained. Results of Ames test on all three systems are negative. None of the systems from the screening studies showed any toxicity on the Mouse Quantal Assay. However, all three systems were toxic to Daphnia. Median effective concentrations (EC_{50}) for the 48 hrs. test were 25.5%, 9.28% and 0.91%, respectively for the TCB, BP and OPP systems. According to EPA evaluation criteria for level 1 biological tests,[6] toxicity of the TCB system is classified as moderate, whereas, those of the BP and OPP systems are classified as extremely high.

TABLE XXII. DYEBATH COMPOSITION - TRICHLOROBENZENE (TCB) SCREENING

Additive		Bath A		Bath B		Bath C
Commercial Dye (yellow 23), mg.		162.57		161.67		159.29
Commercial Carrier (TCB), g.		3.52171		3.50974		3.49707
Wetting Agent (Amaterg T), mg.		86.56		89.92		97.00
Total bath liquor, ml.		1,000		1,000		1,000
Fabric wt., g.	A-1	5.7685	B-5	5.7420	C-9	5.7494
	2	5.6770	6	5.6612	10	5.7399
	3	5.8137	7	5.7878	11	5.6333
	4	5.7521	8	5.7729	12	5.6321
	Total	23.0113		22.9639		22.7547

TABLE XXIII. DYEBATH COMPOSITION - BIPHENYL(BP) SCREENING

Additives		Bath A		Bath B		Bath C
Commercial Dye (Yellow 23), mg.		161.30		162.27		161.28
Commercial Carrier(BP), g.		3.49477		3.49741		3.49733
Wetting Agent(Amaterg T), mg.		92.07		92.60		92.53
Total Bath Liquor, ml.		1,000		1,000		1,000
Sample (Fabric) Weight, g.	A-1	5.7317	B-5	5.8413	C-9	5.6863
	2	5.8794	6	5.8032	10	5.8344
	3	5.7899	7	5.8851	11	5.7339
	4.	5.6036	8	5.6724	12	5.7932
	Total	23.0046		23.2020		23.0478

Table XXIV. DYEBATH COMPOSTION - O-PHENYL PHENOL(OPP) SCREENING

Additives		Bath A		Bath B		Bath C
Commercial Dye (Yellow 23), mg.		159.90		158.81		164.92
Commercial Carrier (OPP), g		3.49477		3.48160		3.49439
Wetting Agent(Amaterg T), mg.		91.00		90.48		94.56
Total Bath Liquor, ml.		1,000		1,000		1,000
Sample (Frabic) Weight, g.	A-1	5.5743	B-5	5.8348	C-9	5.7654
	2	5.8778	6	5.4589	10	5.9624
	3	5.6848	7	5.6328	11	5.8366
	4	5.7568	8	5.7954	12	5.9948
	Total	23.8937		22.7219		23.5592

Carrier Analyses

Liquid (dyebath) Samples --

Results of HPLC determinations of carrier contents in the spentbaths (after dyeing) of the screening studies are shown in Tables XXV and XXVI, respectively for TCB and BP systems. Determinations were made on the spentbath from each of the dyeing, the mixed spentbath (sample for bioassay) and each of the scouring baths. For the TCB system, the mixed spentbath contained 64 mg/l of 1,2,4 TCB, this is essentially the same as the averaged values of the three individual spentbaths. However, variation among the three spentbaths was quite high, this is probably due to the high volatitility of 1,2,4 TCB; thus slight change in dyeing conditions may result in significant variation on the amount of TCB volatilized during dyeing. As can be seen from Table XXV, the amount of 1,2,4 TCB left in both the spentbath and scouring bath accounted for less than 10% of original 1,2,4 TCB content in the dyebath.

For the BP system, BP content in the mixed bath was analyzed as 26.5 mg/l, and the averaged values of the three individual spentbaths was 27.7 mg/l. Similar to the TCB system, BP content in both the spentbath and scouring bath accounted for only a very small fraction of the original BP content in the dyebath.

o-Chlorotoluene -- Trace amount (<10 mg/l) of o-chlorotoluene (OCT) was detected in each of the BP spentbaths. This is expected due to high volatility of OCT (mp. 159°C).

TABLE XXV. DYEBATH ANALYSIS FOR 1,2,4 TCB (SCREENING)

Bath	Conc. mg/l.[b]	% of Original TCB
Spent bath[a]		
A	92 ± 3	3.7
B	57 ± 3	2.3
C	53 ± 3	2.2
	Avg. 67 ± 21	
Mixed[c]	64 ± 3	
Scouring bath[a]		
A	46 ± 4	1.9
B	63 ± 3	2.5
C	71 ± 3	2.9
	Avg. 60 ± 13	

[a] Total both vol. = 1,000 ml.

[b] Average of at least three (3) HPLC determinates

[c] Mixture of Baths A,B & C

TABLE XXVI. DYEBATH ANALYSIS FOR BIPHENYL(BP) (SCREENING)

Bath		BP Conc. Detected, mg/l.[b]	% of Original BP
Spent bath[a]			
	A	16.3 ± 1.3	1.8
	B	43.3 ± 0.9	4.8
	C	23.6 ± 0.8	2.6
	Average	27.7 ± 7.3	
Mixed[c]		26.5 ± 1.4	
Scouring Bath[a]			
	A	7.4 ± 0.4	0.8
	B	6.0 ± 0.4	0.7
	C	4.5 ± 0.1	0.5
	Average	6.0 ± 1.5	

[a] Total Bath Vol. = 1,000 ml.

[b] Average of at least three (3) HPLC determinations

[c] Mixture of Baths A, B & C

Fabric Samples --

Results of carrier analyses on the dyed fabrics (both wet samples from dyebath and scoured and dried samples) are shown in Tables XXVII and XXVIII, respectively for TCB and BP systems. For the TCB system, there was significant difference in the 1,2,4 TCB content between the wet and the dried samples. Scouring, heat-curing and drying are expected to remove majority of TCB from the fabric. However, for the BP systems, difference was quite small, in all cases. Amount of BP on the wet fabric samples was extremely low, compared to the TCB system. Commercial formulation of BP carrier contained 25.8% of BP, whereas that of TCB contained 70% of 1,2,4 TCB. Therefore, BP concentration in the original dyebath was about 0.9 g/l, compared to 2.5 g/l of 1,2,4 TCB in the TCB system. The much lower BP concentration in the dyebath may be responsible for the lower BP on the wet fabric sample. Scouring and drying alone removed a small fraction (about 20%) of the BP on the wet fabric samples.

Mass Balance --

Mass balance of carrier (1,2,4 TCB or BP) in each system was carried out by calculating the amount of 1,2,4 TCB or BP after dyeing, as the sum of the amount in the spentbath and that on the fabric samples. Amount on the fabric samples was again calculated as the amount analyzed on the wet fabric samples in mg/g fabric multiplied by the total fabric weight in the bath. The amount analyzed after dyeing was compared to that initially in the dyebath. As shown in Table XXIX, the amount of 1,2,4 TCB accounted for after dyeing was 46.7% of the initial amount, whereas, the amount of BP accounted for was only 35.5% of

TABLE XXVII. FABRIC ANALYSIS FOR 1,2,4 TCB* (SCREENING)

Fabric #	Fabric wt.g.	1,2,4 TCB Conc., mg/l	Total TCB*, mg	TCB*/Fabric wt., mg/g.
Wet from Dyebath				
A-1	5.7685	566 ± 20	283	49.1
A-3	5.8137	550 ± 20	275	47.3
				48.2
B-5	5.7420	504 ± 54	252	43.9
B-7	5.7878	580 ± 35	290	50.1
				47.0
C-9	5.7494	552 ± 17	276	48.0
C-10	5.7399	502 ± 38	251	43.7
				45.9
			Overall:	47.0 ± 2.7
Scoured (Dried)				
A-4	5.7521	39 ± 5	20	3.5
B-6	5.6612	22 ± 3	11	1.9
C-11	5.6333	19 ± 1	10	1.8

*Extracted with 500 ml. of $MeCl_2$

TABLE XXVIII. FABRIC ANALYSIS FOR BIPHENYL (SCREENING)*

Fabric	Fabric Weight, g.	BP Conc., mg/l**	Total BP, mg.	BP/Fabric, mg/g.
Wet from Dyebath				
A-1	5.7317	144.0	72.0	12.6
A-4	5.6036	140.8	70.4	12.6
			Average	12.6
B-6	5.8851	170.6	85.3	14.5
B-8	5.6724	161.5	80.8	14.2
			Average	14.4
C-9	5.6863	125.9	63.0	11.1
-10	5.8344	127.5	63.8	10.9
			Average	11.0
Scoured (Dried)				
A-2	5.8794	124.5	62.3	10.6
B-5	5.8413	146.5	73.3	12.5
C-11	5.7339	111.0	55.5	9.7

*Extracted with 500 ml of methylene chloride

**Average of three (3) HPLC determinations

TABLE XXIX. MASS BALANCE OF CARRIER (SCREENING)

Dyebath	Initial Carrier, mg*	Carrier Analyzed, mg			% of Initial
		Spentbath	Fabric**	Total	
TCB System					
A	2,458.0	92.0	1,109.1	1,201.1	49.0
B	2,450.0	57.0	1,079.0	1,136.0	46.0
C	2,441.0	53.0	1,044.0	1,097.0	45.0
				Average	46.7
BP System					
A	901.7	16.3	289.9	306.2	34.0
B	902.3	43.3	334.1	377.4	41.8
C	902.3	23.6	253.5	277.1	30.7
				Average	35.5

*carrier content in the commercial formulation: TCB = 69.8% BP = 25.8%

**carrier content on the fabric(mg/g) x (total fabric wt. per bath)

the initial amount. These results suggest that the majority (more than 50% in both systems) of carrier was lost during dyeing through volatilization.

Dye Analyses

Liquid (dyebath) Samples --

Results of spectrophotometric determination of dye (yellow 23) content in the spentbaths and scouring baths of screening studies are shown in Tables XXX, XXXI and XXXII, respectively for the TCB, BP and OPP systems. In all three systems, dye content analyzed for the mixed baths was essentially the same as the averaged value of the three bath samples. In all dyebaths, the residual dye content in the spentbath was less than 10% of the original dye in the dyebath, indicating that at least 90% exhaustion was achieved in these dyebaths. Furthermore, variation in dye exhaustion among the three dyebaths was small in all three systems. This is due to consistency of dyeing operation and high accuracy of the analytical technique.

For the scouring baths, dye content was small in all dyebaths, and variation was again small among the baths in each system. This is an indication of good fixation of dye to the fiber during dyeing, due to consistent dyeing operation.

Fabric Samples --

Dyed fabric samples from each of the dyebaths with TCB, BP and OPP systems were extracted with $MeCl_2$, and the extracts were analyzed, spectrophotometrically, for dye (yellow 23) content. Dye content in mg per gram of fabric was calculated for each of the dyed samples, both wet from the dyebath

TABLE XXX. DYEBATH ANALYSIS FOR YELLOW 23 - TCB SCREENING

Dyebath[a]	Avg. Conc., mg/l [b]	% of Original Y-23
A	6.13	9.3
B	4.90	7.5
C	4.76	7.4
	Avg. 5.26	8.1 ± 1.0
Mixed[c]	5.32	
Scouring Bath[a]		
A	2.19	3.3
B	1.70	2.6
C	1.60	2.5
		2.8 ± 0.4

[a] Total bath vol. = 1,000 ml.

[b] 1:1 dilution of dyebath liquor with acetone, at 375 mn, average of three (3) measurements.

[c] Mixture of baths A, B & C.

TABLE XXXI. DYEBATH ANALYSIS FOR YELLOW 23 - BP SCREENING

Dyebath[1]	Y-23 Conc. Detected,[2] mg/l	% of Org. Y-23
A	5.35	8.1
B	5.97	9.0
C	4.85	7.4
	Average 5.39	8.2 ± 0.8
Mixed[3]	5.34	
Scouring Bath		
A	2.72	4.1
B	2.28	3.4
C	1.39	2.1
	Average 2.13	3.2 ± 1.0

1. Total Bath Vol. = 1,000 ml
2. Average of three (3) spectroscopic determinations on 1:1 dilution of bath liquor with acetone
3. Mixture of bath A, B & C

TABLE XXXII. DYEBATH ANALYSIS FOR YELLOW 23 - O-PHENYL PHENOL SCREENING

Dyebath[1]		Conc. Detected,[2] mg/l	% of Org. Y-23
A		3.02	4.7
B		3.09	4.8
C		2.95	4.4
	Average	3.02	4.6 ± 0.2
Mixed[3]		2.98	
Scouring Bath			
A		0.56	0.9
B		0.54	0.8
C		0.61	0.9
	Average	0.57	0.9 ± 0.1

1. Total Bath Vol. = 1,000 ml.

2. Spectroscopic determination at 375 nm on 1:1 dilution of bath liquor with acetone, average of three (3) determinations.

3. Mixture of baths A, B & C.

and those scoured and dried. The results are tabulated in Tables XXXIII, XXXIV, and XXXV, respectively for TCB, BP and OPP systems. Consistently in all cases, variations of fabric dye contents are small among samples in the same bath, and among samples in different baths of the same system. Furthermore, the difference between dye contents on the wet samples and scoured samples were small in all cases. This is another indication of consistent dyeing operation and good dye fixation. Dye contents of fabric from BP and OPP systems were slightly higher than those of samples from the TCB system, suggesting that TCB is a slightly less efficient carrier, as compared to BP or OPP. Results of dye analyses on all three screening systems are summarized in Table XXXVI.

Mass Balance --

Based on the above results of analyses, a mass balance on the dye in each of the dyebath systems was made.

Two different ways of balancing the mass of dye were attempted. As shown in Table XXXVII, the initial mass of yellow 23 in the bath was calculated from the weight of initial commercial dye in the bath and the yellow 23 content in the commercial formulation. The mass of yellow 23 after dyeing was assumed to be the sum of yellow 23 left in the bath liquor and those picked up by the fabric, calculated as the product of dye content on wet fabric and the total weight of fabric dyed in the bath. For all baths, consistently, over 92% of the initial yellow 23 was accounted for. In another way of balancing the yellow 23 in the bath (Table XXXVIII), mass of yellow 23 after dyeing was assumed as the sum of the yellow 23 left in the bath liquor, those analyzed

Table XXXIII. Fabric Analysis for Yellow 23 - TCB Screening*

Fabric # Wet from Dyecloth	Initial Fabric wt., g.	Total Y-23, mg.	Y-23/Fabric, mg/g.
A-1	5.7685	14.03	2.43
A-3	5.8137	14.82	2.55
			2.49
B-5	5.7420	13.62	2.37
B-7	5.7878	14.10	2.44
			2.41
C-9	5.7494	13.88	2.41
C-10	5.7399	13.71	2.39
			2.40
Scoured (Dried)			
A-4	5.7521	13.34	2.32
B-6	5.6612	12.83	2.27
C-11	5.6333	12.87	2.29

*extraction with 500 ml. of $MeCl_2$

Table XXXIV. Fabric Analysis for Yellow 23 - BP Screening*

Fabric	Weight, g	Total Y-23, mg.		Y-23/Fabric, mg/g
Wet from Dyebath				
A-1	5.7317	15.33		2.67
-4	5.6036	15.00		2.68
			Average	2.68
B-6	5.8851	15.63		2.66
-8	5.6724	15.22		2.68
			Average	2.67
C-9	5.6863	15.44		2.71
-10	5.8344	15.77		2.70
			Average	2.71
Scoured (Dried)				
A-2	5.8794	14.18		2.41
B-5	5.8413	14.28		2.44
C-11	5.7339	14.88		2.59

*Extraction with 500 ml of methylene chloride.

Table XXXV. Fabric Analysis for Yellow 23 - OPP Screening*

Fabric	Weight, g	Total Y-23, mg.	Y-23/Fabric, mg/g
Wet from Dyebath			
A-1	5.5743	14.31	2.57
-2	5.8778	14.74	2.51
			Average 2.54
B-6	5.4589	13.82	2.53
-7	5.6328	15.27	2.71
			Average 2.62
C-11	5.8366	15.60	2.67
-12	5.9948	16.41	2.74
			Average 2.71
Scoured (Dried)			
A-3	5.6848	14.58	2.55
B-5	5.8348	14.91	2.56
C-9	5.7654	14.40	2.50

* extraction with 500 ml of methylene chloride.

Table XXXVI. Summary of Dye Analysis in the Screening Studies

	TCB Bath	BP Bath	OPP Bath
% Dye Exhaustion	91.9	91.9	95.4
Averaged Residual Y-23 in Spentbath, mg/l*	5.26 (5.32)	5.39 (5.34)	3.02 (2.98)*
Averaged Residual Y-23 in Scourbath, mg/l	1.83	2.13	0.57
Averaged Y-23 on Fabric, mg/g			
Wet	2.43	2.68	2.62
Scoured and Dried	2.29	2.48	2.54

* values in parentheses are those of mixed baths.

Table XXXVII. Mass Balance of Yellow 23 (I) - Screenings

Dyebath	Initial y 23, mg*	Yellow 23 Analyzed, mg			% of Initial
		Spent Bath	Fabric**	Total	
TCB System					
A	65.84	6.13	57.30	63.43	96.3
B	65.48	4.90	55.34	60.24	92.0
C	64.51	4.76	54.61	59.37	92.0
BP System					
A	65.81	5.35	61.65	67.00	101.8
B	66.21	5.97	61.95	67.92	102.6
C	65.80	4.85	62.46	67.31	102.3
OPP System					
A	65.24	3.02	60.60	63.71	97.7
B	64.79	3.09	59.53	62.62	96.7
C	67.29	2.95	63.85	66.80	99.3

* yellow 23 content in the commerical dye = 40.5%
**yellow 23 content on wet fabric (mg/g fabric) x (total fabric wt. per bath)

Table XXXVIII. Mass Balance of Yellow 23 (II) - Screenings

Dyebath	Initial[1] y-23, mg	Yellow 23 analyzed, mg					% of Initial
		Spent Bath	Fabric (wet)	Fabric (dry)[2]	Scour Bath	Total	
TCB System							
A	65.84	6.13	28.85	26.52	2.19	63.69	96.7
B	65.48	4.90	27.72	25.96	1.71	60.35	92.2
C	64.51	4.76	27.59	25.80	1.60	59.75	92.6
BP System							
A	65.81	5.35	30.66	28.12	2.72	66.85	101.6
B	66.21	5.97	3.085	28.61	2.28	67.71	102.3
C	65.80	4.85	31.21	29.86	1.39	67.31	102.3
OPP System							
A	65.24	3.02	29.05	29.18	0.56	61.84	94.7
B	64.79	3.09	29.09	29.77	0.54	62.49	96.5
C	67.29	2.95	32.01	29.32	0.61	64.89	96.4

[1] yellow 23 content in the commercial dye = 40.8%

[2] yellow 23 content on the dried fabric (mg/g fabric) x (wt. of two dried fabrics)

from the fabric samples (both wet and dry) and those analyzed in the scouring liquor. The values obtained from the three baths are essentially identical to those obtained with the first method. Furthermore, the differences in yellow 23 content between the wet and the dry pick-ups (amount removed during scourings) is identical, in each case, to that analyzed in the scouring bath.

Summary of Screening Studies

Results of the screening studies, including bioassays, carrier and dye contents of the samples (spentbaths) are summarized in Table XXXIX. All three systems showed significant toxicity on the Daphnia magna assay. Both Ames and Mouse Quantal assays gave negative results on all three systems. Consequently, in the in-depth studies (TCB and BP systems), only Daphnia magna assay was used.

Results of carrier analysis in these systems showed that a significant amount of carrier and other dyebath additives (o-chlorotoluene) was lost, through volatilization, to the environment.

Results of dye analysis in these systems indicated that dyeing procedure and operation in the screening studies was consistent with the industrial practice. Good dyebath exhaustion and dye/fiber fixation were achieved in all dyebaths.

Table XXXIX. Summary of Screening Studies - Bioassay

	TCB System	BP System	OPP System
Spentbath Contents:			
Dye (yellow 23), mg/l	5.32	5.34	2.98
Carrier, mg/l	63.0 (1,2,4 TCB)	41.9 (BP)	--[a]
Bioassay Results:			
Ames (w/ and w/o liver microsomal enzyme)	Negative	Negative	Negative
Mouse Quantal Assay	Nontoxic	Nontoxic	Nontoxic
Daphnia magna Assay [b]			
EC_{50}, %			
24 hrs.	31.2(24.3 - 38.1)	20.45(8.16-51.25)	0.91(0.70 - 1.12)
48 hrs.	25.5(18.2 - 32.7)	9.28(7.74-10.83)	0.91(0.65 - 1.18)

[a] analysis not made

[b] values in parentheses are 95% confidence interval

IN-DEPTH STUDIES

From the results of screening studies, in-depth study was then carried out on the TCB and BP systems. The objectives of the in-depth study are to identify the component(s) responsible for the toxicity observed in the screening study and to delineate any synergistic or antagonistic effect in the system. To minimize the number of bioassay testings, it was decided that the major components (carrier and dye) would be analyzed first, and the combined effect of the major components would be estimated and compared with the result from the whole dyebath (screening study).

TCB System

Due to the low solubility of TCB and yellow 23 in water, it was not possible to analyze these components alone. The commercial formulations containing the carrier and emulsifier and the dye and dispersing agent were analyzed. The emulsifier and the dispersing agent were then analyzed alone, and the toxicities of the pure carrier (1,2,4 TCB) and pure dye (yellow 23) calculated. Table XL summarizes the bioassays carried out and their results. Two significant conclusions were drawn from these bioassay results;

1) Emulsifier (Test TCB-5A) in the commercial carrier is essentially nontoxic, therefore, the toxicity observed in Test TCB-3A (commercial carrier) is the toxicity of TCB (1,2,4 TCB), and
2) the dispersing agent initially used in the experiments (Test TCB-6A and Test TCB-7A) was that reprocessed from the commercial dye formulation (by extracting yellow-23 dye with acetone). Test TCB-6A showed extreme toxicity, whereas, Test TCB-7A (solution was brought to boiling through mock dyeing) showed marked decrease in toxicity. It was suspected that the chemical used might be contaminated with the extraction solvent - acetone which is known to show extreme

Table XL. Summary of Bioassay(Daphnia magna) Results - TCB(In-Depth Study)

Test Number	Sample	Preparation	Concentration of Component Analyzed, mg/l	EC_{50}(48 hr.)[a]
TCB - 1B	dyebath discharge (spentbath)	actual dyeing	1,2,4 TCB = 63.8 y-23 = 5.32	25.5% (18.2 - 32.7%)
- 1A	commercial carrier	room temperature	1,2,4 TCB = 76.3	55.4% (41.2 - 69.9%)
- 2A	commercial dye	room temperature pH. adjusted	y-23 = 5.36	41.4% (31.2 - 51.6%)
- 3A	commercial carrier (repeat of -1A)	room temperature	1,2,4 TCB = 63.0	68.3% (50.6 - 86.0%)
- 4A	commercial dye & carrier	room temperature pH. adjusted	1,2,4 TCB = 63.0 y-23 = 5,61	63.7% (37.3 - 90.1%)
- 4A - 2	repeat of -4A	same as -4A	1,2,4 TCB = 63.9 y-23 = 7.56	66.0% (51.7 - 80.4%)
- 5A	emulsifier	room temperature	total = 6.8	87.0%(not toxic)
- 6A	dispersing agent[b]	room temperature pH. adjusted	total = 8.9	9.1% (6.5 - 12.6%)
- 7A	dispersing agent[b]	mock dyeing[c] pH. adjusted	total = 9.8	59.4% (46.5 - 72.3%)
- 8A	dispersing agent[d]	room temperature	total = 9.5	non-toxic
- 9A	dispersing agent[d]	mock dyeing[c] pH. adjusted	total = 9.5	non-toxic

[a]values in parenthesis are 95% confidence interval. [b]reprocessed from commercial dye formulation.
[c]dyeing procedure followed, without any other additives or fabric. [d]Reax 83A, commercial dispersing agent.

toxicity in Daphnia assay. The supplier of the commercial dye was contacted and sample of dispersing agent (Reax 83A) used in the dye formulation was obtained. The bioassays were repeated (Test TCB-8A and Test TCB-9A). Both samples showed no toxicity. It was then concluded that the toxicity initially observed was due to contamination with acetone, and that the toxicity observed in the commercial dye formulation (Test TCB-2A) was due to that of the yellow 23 dye.

Other dyebath additives which have not been tested individually are the wetting agent and the acetic acid. However, no significant toxicity contributions are expected from these two chemicals. The wetting agent is a surfactant similar to the emulsifier and dispersing agent used in the formulations. Acetic acid was used to adjust the pH of the dyebath liquor. All bioassay samples, with or without pH adjustment have pH range of 4.5-6.

It was, therefore, assumed that the toxicity observed in the dyebath liquor was contributed predominately by 1,2,4 TCB and yellow 23 dye. Based on the above assumption, attempts were made to correlate the toxicity of the dyebath sample with those of the individual components. Table XLI shows the concentrations of each component and the EC_{50}, in concentration term, in these dyebath samples. EC_{50} in concentration term was calculated from the concentration and percent EC_{50} of the sample.

A standard method[11] for assessing additive toxicity of chemical mixtures was used to estimate the additive effect of individual components in the mixture (dyebath samples). The equation used was:

$$S = (I_m/I_i)$$

Table XLI Concentrations and EC_{50}(48 hr.) of Key Dyebath Samples - TCB

Sample	1, 2, 4 TCB		Yellow 23	
	conc., mg/l	EC_{50}, mg/l	con., mg/l	EC_{50}, mg/l
TCB-1B dyebath EC_{50}=25.5%	63.8	16.3	5.32	1.36
TCB-1A carrier EC_{50}=55.4%	76.2	42.3	-	-
TCB-2A dye EC_{50}=41.4%	-	-	5.36	2.22
TCB-3A carrer EC_{50}=68.3%	63.3	43.2	-	-
TCB-4A carrier + dye EC_{50}=63.7%	63.0	40.1	5.61	3.57
TCB-4A-2 carrier + dye EC_{50}=66.0%	63.9	42.2	7.56	4.99

where

S = sum of biological activity, and

I_i and I_m = toxicities (EC_{50}s) of the individual chemicals and mixtures, respectively

If the sum of biological activity, S, is 1.0, the toxicity is simple additive. Sums that are less than 1.0 indicated greater than additive (synergistic) toxicity, and sums greater than 1.0 indicate less than additive (antagonistic) toxicity.

Since both the emulsifier and dispersing agent in the commercial carrier and dye formulations showed no toxicity. The toxicities of 1,2,4 TCB and pure yellow 23 dye were assumed to be the same as those of commercial carrier (TCB-1A, -3A) and commercial dye (TCB-2A), respectively. The EC_{50}s in concentration term were calculated as shown in Table XLI. These values were then used to estimate the additive effect, S, in the dyebath (TCB-1B).

For the total dyebath liquor (TCB-1B);

$$S = [(TCB)_{1B}/(TCB)_{3A}] + [(t\text{-}23)_{1B}/(y\text{-}23)_{2A}]$$
$$= (16.3/43.2) + (1.36/2.22)$$
$$= .38 + .61$$
$$= .99$$

The results indicate that the toxicity observed in this system (TCB-1B) is essentially the additive effect of 1,2,4 TCB and yellow 23 dye.

BP System

A similar procedure as used in the in-depth study of the TCB system was followed. An aqueous sample containing commercial BP carrier (Carolid RNO) alone was prepared for Daphnia magna assay. Due to variation of Daphnia

results on the two initial samples, additional samples were prepared and tested. Sample BP-1A-4 was delayed when transfered from Litton Bionetics to Battelle, and was not tested within 24 hrs., as required by the protocol. Therefore, the result of this test was ignored. There is still considerable variation among the results of the other three samples (Table XLII) although 95% confidence intervals overlap among the three sets of results.

Due to low solubility of BP in water, it was not possible to prepare aqueous BP sample at the concentration needed. Instead, aqueous sample containing commerical OCT solvent (Halso 99) used for formulation of Carolid RNO was prepared and tested (BP-2A). Bioassay and analytical results of all BP samples are shown in Table XLII. Due to the special pattern (there were no partial responses between 100% and 0% survival) of observed toxicity on the BP-2A sample, exact EC_{50} of the sample could not be calculated. The EC_{50} for this sample was estimated to be between 50 and 100%, and more likely to be much larger than 50%. Therefore, toxicity observed in the BP-1A samples was assumed to be due mainly from BP. EC_{50} values in terms of concentration can then be calculated, as shown in Table XLIII.

Using EC_{50} values in Table XLIII and EC_{50} of yellow 23 previously obtained (Table XLI), additive effect in the BP system was estimated with the following equation:

$$S = [(BP)_{1B}/(BP)_{1A}] + [(y\text{-}23)_{1B}/(y\text{-}23)_{2A}]$$

where, $(BP)_{1B}$= 3.89 mg/l, $(y\text{-}23)_{1B}$ = 0.50 mg/l (Table XLIII)

and, $(y\text{-}23)_{2A}$= 2.22 mg/l (Table XLI).

Table XLII. Summary of Bioassay Results - BP (In-Depth Study)

Test No.	Sample	Preparation	conc. of Component analyzed, mg/l	48hr. Daphnia, EC_{50},* %
BP-1B	dyebath, discharge (spentbath)	actual dyeing (screening)	BP=41.9, OCT=7.4 Y-23=5.34	9.28 (7.74 - 10.83)
-1A	commercial carrier	rm. temp.	BP=25.6 OCT=39.9	23.32 (17.56 - 29.08)
-1A-2	commercial carrier	rm. temp.	BP=31.4 OCT=57.6	17.06 (13.71 - 20.42)
-1A-3	commercial carrier	rm. temp.	BP=27.4 OCT=65.5	27.1 (28.20 - 72.10)
-1A-4	commercial carrier	rm. temp.	contents not analyzed	50.2 (28.20 - 72.10)
-2A	commercial OCT (Halso 99)	rm. temp.	OCT=27.7	>>50**

* values in parenthesis are 95% confidence interval.

** EC_{50} undetermined because of pattern of observed toxicity.

Table XLIII. Concentrations and EC_{50} of Key Dyebath Samples - BP

Sample	BP		OCT		Yellow 23	
	conc., mg/l	EC_{50}, mg/l	conc., mg/l	EC_{50}, mg/l	conc., mg/l	EC_{50}, mg/l
BP-1B dyebath EC_{50}=9.28%	41.9	3.89	(7.4)*	-	5.34	0.50
BP-1A carrier EC_{50}=23.32%	25.6	5.97	39.9	9.30	-	-
BP-1A-2 carrier EC_{50}=17.06%	31.4	5.36	57.6	9.83	-	-
BP-1A-3 carrier EC_{50}=27.10%	27.4	7.43	65.5	17.75	-	-
BP-2A OCT (Halso 99) EC_{50} >>50%	-	-	27.7	-	-	-

*Trace Amount

Using the EC_{50} values for BP obtained from Tests BP-1A, BP-1A-2 and BP-1A-3 (Table XLIII), the sum of the biological activity, S, for the BP system was calculated, using the above equation, as 0.89, 0.97 and 0.76. Taking into consideration the uncertainty of the test results (95% confidence interval), the above three values for S are not significantly different from 1. Therefore, it was concluded that the toxicity observed in the BP spentbath sample was due mainly from the dye (yellow 23) and biphenyl(BP) present, and there was no synergistic or antagonistic effect observed in this system.

IMPROVEMENT OF THE TOXIC TCB SYSTEM

The major approach to reduce toxicity is to reduce the concentration of both the dye (yellow 23) and TCB in the spentbath by 1) varying dyeing time and, 2) varying original carrier content to achieve maximum dyebath exhaustion. Dyeing time was varied from 30 min. (boiling) to 90 m[illegible] as shown in Table XLIV. However, dyebath exhaustion and residual dye concentration in the spent bath did not show any significant improvement. The carrier concentration in the bath was held constant at 3.5 g/l in all these dyebaths. Results in Table XLIV, indicate that maximum exhaustion has been reached at 30 min. boiling. However, in order to achieve good leveling, 45 min. boiling (standard dyeing time used in this study), was continued to be used.

Carrier concentration in the dyebath was reduced from 3.5 g/l, to 2.0 g/l. Results shown in Table XLV indicate that at 2.0 g/l, slightly better exhaustion was obtained and significantly low residual TCB and dye concentrations were detected. At 2.0 g/l carrier concentration, the estimated EC_{50} can be calculated by assuming that the toxicity of the bath sample is additive of the individual toxicities of carrier and dye (discussed in detail earlier).

XLIV. EFFECTS OF VARYING DYEING TIME ON THE TCB SYSTEM

Dyeing time mins.	Wt. of * carrier, g.	Wt. of * dye, mg.	Exhaustion %	Residual dye, mg/l
30	3.49800	175.05	92.9	5.07
	3.50223	175.89	94.0	4.31
			93.5	4.69
45	3.50523	174.63	93.3	4.77
	3.50248	174.72	93.8	4.42
			93.6	4.60
60	3.50116	174.52	93.1	4.91
	3.49606	174.95	94.1	4.21
			93.6	4.56
75	3.50100	174.63	94.8	3.70
	3.50204	175.99	95.1	3.52
			95.0	3.61
90	3.49823	173.20	94.3	4.08
	3.50080	175.85	94.7	3.80
			94.5	3.94

*total bath volume = 1.0 liter

XLV. EFFECTS OF VARYING DYEBATH CARRIER CONCENTRATION ON THE TCB SYSTEM[1]

Wt. of carrier,[2] g.	Wt. of dye,[2] mg.	Dye Exhaustion %	Residual concs. in Spent Bath, mg/l. 1,2,4 TCB	yellow 23
--	175.76	88.0	--	8.61
--	176.10	88.9	--	7.98
--	175.93	86.4	--	9.76
		87.8		8.78
2.00473	175.27	95.4	--	3.29
2.00042	175.94	95.1	24.6	3.52
2.00081	174.85	95.4	21.2	3.28
		95.3	22.9	3.36
3.52171[3]	162.57	91.7	92.0	6.13
3.50974[3]	161.67	92.5	57.0	4.90
3.49707[3]	159.29	92.6	53.0	4.76
		92.0	68.0	5.26
3.50523	174.63	93.3	--	4.77
3.50248	174.72	93.8	--	4.42
		93.6		4.60

[1] dyeing time = 45 mins.

[2] total bath volume = 1.0 liter

[3] TCB-1B

The estimated EC_{50} is 48.9% (Table XLVI) as compared to 25.5% for the TCB-1B.

The above results indicate that the toxicity of the TCB system can be reduced from high to moderate toxicity by increasing the efficiency of the dyebath and dyeing operation. Although no experimental work was carried out for the BP and OPP systems, it is reasonable to expect that a similar approach can be used to reduce the toxicity of these systems.

XLVI. CALCULATED TOXICITY FOR THE IMPROVED TCB SYSTEM (2.0 g/l TCB)

Bath	EC_{50},%	1,2,4 TCB conc., mg/l	yellow 23 conc., mg/l
TCB-1B	25.5	63.8	5.32
-2A(dye)	41.4	--	5.36
-3A(carrier)	68.3	63.3	--
Current	--	22.9	3.36

$$S = \Sigma(I_m/I_i)$$

S = sum of biological activity (EC_{50})

I = biological activity (EC_{50}) of individual chemicals or mixtures

i & m = individual and mixture

for TCB-1B, $S = \frac{(63.8)(.255)}{(63.3)(.683)} + \frac{(5.32)(.255)}{(5.36)(.414)} = .99$

for current bath, $S = 1 = \frac{(22.9)(X/100)}{(63.3)(.683)} + \frac{(3.36)(X/100)}{(5.36)(.414)}$

X = expected EC_{50},%

= 48.9

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